EPA Registration No. 84610-2 vol. 1

NANOSILVA, LLC

September 23, 2013

Mr. Jed Costanza
One Potomac Yard
2777 Crystal Drive
Arlington, VA. 22202

Subject: Study Submission

Dear Jed,

Per your guidance, we have completed the revisions to the study entitled "The Quantification and Characterization of Silver Released from Textiles Treated with NSPW-L30SS As a Result of Simulated Laundering Conditions". As requested, we have combined the two previously submitted studies entitled "The Quantification and Characterization of Silver Released from Textiles Treated with Nanosilva (NSPW-L30SS) as a result of washing" and "The Quantification of Silver Released from Textiles Treated with NSPW-L30SS as a Result of Simulated Contact/Exposure Conditions with Synthetic Saliva" into one study as submitted herein.

In closing, we would like to thank you for your time and consideration regarding this submission. Your guidance was invaluable and much appreciated.

Regards,

NanoSilva, LLC.

Wayne Krause
V.P. Operations

TRANSMITTAL DOCUMENT

Name and Address of Submitter: Nanosilva, LLC
C/O Wayne Krause
272 Brandon Ln.
Newnan, GA. 30265

Regulatory action in support of which this package is submitted:
Application for Registration of Nanosilva™ Antimicrobial [Silver-Silica Colloid-(NSPW-L30SS-Product Code)]

EPA Reg. No. /File Symbol: N0. 84610-~~XX~~ E

Alternative Test Material Names: Covalently bonded Silver-Silica Colloid in aqueous solution

Transmittal Date: September 23, 2013

Administrative Materials		
1	Transmittal Document Cover Letter	
Volume No.	**Citation**	**MRID No.**
2	The Quantification and Characterization of Silver Released from Textiles Treated with NSPW-L30SS As a Result of Simulated Laundering Conditions; Non-Guideline Study No. 110112.0001 Revision 3, Wayne Krause, 2013 Unpublished Study by Nanosilva, LLC, 353 pages with Confidential Attachment. 3 Copies	49224901

Company Official: Wayne Krause, Vice President Operations
Company Name: NanoSilva, LLC.
Company Contact: Wayne Krause, (678) 593-8131, wkrause63@hotmail.com

Signature: Wayne J Krause

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

August 30, 2013

NanoSilva, LLC
2811 NE 14th Street
Ocala, FL, 34470

Attention: Wayne Krauss

Subject: Review of Results from Studies to Determine the Amount and Size Distribution of Silver Released from Textiles Treated with Nanosilva (NSPW-L30)

On March 8, 2013 Nanosilva LLC submitted the following two study reports concerning the release of silver from textiles treated with Nanosilva:

1. The Quantification and Characterization of Silver Released from Textiles Treated with NanoSilva (NSPW-L30) as a Result of Washing
2. The Quantification of Silver Released from Textiles Treated with NSPW-L30SS as a Result of Simulated Contact/Exposure Conditions with Synthetic Saliva

EPA reviewed these two studies and in an April 12, 2013 letter we detailed major revisions that were required to be completed before the study results could be used in reaching a decision regarding your application to register Nanosilva for use as a textile preservative. On May 23, 2013 you sent me an email with one revised study report concerning the release of silver from textiles treated with Nanosilva:

1. The Quantification and Characterization of Silver Released from Textiles Treated with NSPW-L30SS: As a Result of Simulated Laundering Conditions. Study Number 110112.0001 REV 2

This revision largely addressed the concerns we detailed in our letter of April 12, 2013. However, we have identified the following concerns that need to be addressed before we can accept this study.

1. Use of MDL instead of LOQ in Tables 11, 12, 13, and 17.

In Section 8.1.2 the method detection level (MDL) for silver is stated as 0.0094 µg/L for liquid samples and 0.94 µg/kg for solid samples. In Section 8.1.3 you state that the limit of quantitation (LOQ) for silver in water samples is 0.094 µg/L and 9.4 µg/kg for solid samples. The LOQ is defined in Section 7.2.3 as being 10 times the MDL, which was previously communicated to you in a phone call on January 25, 2013.

Tables 11, 12, 13, and 17 state that the MDL, not the LOQ, was used in determining if the reported silver concentration was detectable. Please correct these tables so that no concentrations below the LOQ are reported.

2. Table 5 indicates that textiles used in the saliva test were 8 by 20 cm in size. However, the text indicated that 10 by 22 cm sized swatches were used for the saliva test. Please revise Table 5 to indicate the size of textiles used in the saliva test.

3. Please revise Table 7 to indicate that the standard concentration has units if µg/L.

4. Please provide a description of the type of dye and how it was applied to each shirt. Please provide the diameter of yarn used to prepare the shirts.

Please make the above revisions to the Study Number 110112.0001 REV 2 and mail the final version to EPA's Document Processing Desk on or before September 26, 2013.

Sincerely,

Jed Costanza, Ph.D.
Regulatory Management Branch 1
Antimicrobials Division (7510P)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: August 27, 2013

SUBJECT: **Nanosilva:** Summary of Hazard and Science Policy Council (HASPOC) Meetings on June 20 and August 15, 2013: Recommendations on Data Requirements and Waivers for Nanosilva.

PC Code: 072599
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0056700
MRID No.: N/A

DP Barcode: N/A
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: N/A
40 CFR: NA

FROM: Julie Van Alstine, MPH
Executive Secretary, HASPOC
Health Effects Division (HED; 7509P)

THROUGH: Jess Rowland, Co-Chair
Anna Lowit, Ph.D., Co-Chair
Hazard and Science Policy Council (HASPOC)
HED (7509P)

TO: Jed Costanza, Ph.D.
Antimicrobial Division (AD; 7510P)

JUNE 20, 2013 MEETING ATTENDEES:

HASPOC Members: Anna Lowit, Elizabeth Mendez, Jeff Evans, Jeff Dawson, Jess Rowland, Jonathan Chen, Michael Metzger, P.V. Shah, Ray Kent

Presenter: Jed Costanza

Other Attendees: Joey Bever, Steve Weiss, Jonathan Leshin, Tim McMahon, Christopher Schlosser, Jaime D'Agostino, Minerva Mercado, Monique Perron, Yung Yang, Monica Hawkins, Tim Leighton, Amber Aranda, Michelle Centra, Jenny Tao, Tim Dole, Uma Habiba, Anwar Dunbar, Kristin Rury, Julie Van Alstine

AUGUST 15, 2013 MEETING ATTENDEES:

HASPOC Members:	Anna Lowit, Elissa Reaves, Elizabeth Mendez, Jeff Dawson, John Kough, Michael Metzger, P.V. Shah, Ray Kent
Presenter:	Timothy Leighton
Other Attendees:	Jonathan Leshin, Timothy Leighton, Joey Bever, Seiichi Murasaki, Kristin Rury, Julie Van Alstine

I. PURPOSE OF MEETING

In August, 2009 Nanosilva LLC submitted an application to register Nanosilva, a nanosilver containing pesticide, as a new active ingredient. Nanosilva LLC submitted acute toxicity studies, a study that determined the amount of silver leaching from plastic coupons incorporating Nanosilva, and a request to waive some of the required toxicity studies. The Antimicrobials Division (AD) requested that the Hazard and Science Policy Council (HASPOC) evaluate two issues: 1) general considerations for toxicity data needs for nanoscale materials and 2) specific data needs for Nanosilva. The HASPOC met on June 20 and August 15, 2013 to discuss all of these issues.

II. GENERAL CONSIDERATIONS FOR NANOSCALE MATERIALS DATA NEEDS

In this memo the HASPOC delineates important considerations for toxicology data needs for nanoscale materials to facilitate a transparent and consistent approach to evaluating pesticides containing nanoscale ingredients. The foundation for these considerations can be found in Appendix A of the HeiQ-AGS 20 Decision Document with further refinements based on insights gained from further review of the toxicology database for nanosilver and on the HASPOC's experience with conventional pesticides. The HASPOC generally uses a risk-based weight of the evidence (WOE) approach in considering toxicology data needs. This WOE approach does not involve a "check-box approach," but instead evaluates and weighs the confidence and uncertainty associated with multiple scientific areas (http://www.epa.gov/pesticides/regulating/part158-tox-data-requirement.pdf). The following four principles used by HASPOC for toxicity needs for conventional pesticides can be applied, with adjustments, to nanoscale materials.

1. **Physical-Chemical Properties, Including Particle Information:** Some important nanoscale properties for consideration are: form (e.g., powder, liquid), composition (i.e., whether or not the material is a composite), size, shape, coating, and surface charge.

2. **Use Pattern and Exposure Scenarios:** Exposure patterns and pathways need to be evaluated and described to ensure that toxicity data match, to the extent possible, the appropriate duration and route of exposure in addition to the population of interest. This description needs to explicitly consider exposure to occupational handlers and consumers separately.

3. **Toxicity Profile:** First, the available experimental toxicology studies need to be summarized for the nanoscale ingredients in a specific pesticide product being evaluated. Then, available, relevant toxicology data on the same nanoscale ingredients from the open literature needs be considered. Uncertainty factors also need to be described.

4. **Margins of Exposure (MOEs) or Other Appropriate Risk Metrics (e.g., Percent Reference Dose):** The WOE approach includes characterization of risk metrics which incorporate toxicity and exposure together. MOEs should be compared to the target MOE and associated confidence and uncertainties need to be described.

III. SUMMARY OF USE PROFILE AND RISK ASSESSMENT FOR NANOSILVA

Nanosilva is a liquid suspension containing 1% nanosilver particulates by weight where the nanosilver active-ingredient is attached to crystalline silica via a thiolate bond. Nanosilva LLC is proposing that Nanosilva be incorporated into plastics and textiles at 30 mg per kg of article treated to suppress the growth of bacteria, algae, fungus, mold, and mildew, which cause odors, discoloration, stains, and deterioration. Nanosilva LLC proposed that Nanosilva be used in a wide range of non-food contact categories including housewares, building materials, bathroom fixtures and accessories, electronics, appliances, personal care products, automotive equipment, hospital and institutional facility equipment, sporting goods, and textiles.

There are no product-specific intermediate- or long-term experimental toxicity studies available for Nanosilva. EPA evaluated the risk from occupational and consumer exposure using experimental toxicology data available in the scientific literature for nanosilver.

Oral and dermal exposure is being assessed using oral toxicity studies by Kim *et al.* (2008) and Kim *et al.* (2010). These studies used CMC coated nanosilver with average diameter of 56 and 60 nm, respectively; EPA acknowledges that the Kim studies use nanosilver which is different from the diameter and surface coating of the nanosilver in Nanosilva. Unlike most toxicity studies from the scientific literature, however, these studies were completed according to Organization for Economic Cooperation and Development (OECD) guidelines. Moreover, these studies identified histopathological patterns in the liver that were indicative of distinct adverse effects. EPA has determined that the no-observed adverse-effect level (NOAEL) of 30 mg/kg/day from the 28-day oral toxicity study by Kim *et al.* (2008) is the point of departure (POD) for short-term oral exposures (<30 days) to the nanosilver in Nanosilva based on significant increases in alkaline phosphatase and cholesterol and significant changes in hematology that were accompanied by histopathological evidence of liver toxicity (bile-duct hyperplasia around central vein, infiltration of inflammatory cells, and dilation of the central vein) seen at the lowest-observed adverse-effect level (LOAEL) of 300 mg/kg/day. EPA has also determined that the LOAEL of 30 mg/kg/day based on histopathological evidences of liver toxicity (bile-duct hyperplasia with focal, multifocal, or lobular necrosis) in both males and females from the 90-day oral toxicity study by Kim *et al.* (2010) is the POD for intermediate-term oral exposures (1 to 6 months) to the nanosilver in Nanosilva. A dermal absorption factor (DAF) of 6.7% based on a human clinical study by Wan *et al.* (1991) is applied to extrapolate from the oral to dermal routes.

EPA has determined that the NOAEL of 49 $\mu g/m^3$ is the POD for short- and intermediate-term inhalation exposures to the nanosilver in Nanosilva. The inhalation toxicity study by Song *et al.* (2012) used uncoated nanosilver with average diameters of 14 to 15 nm, respectively, which is similar to the diameter, but different than the surface coating, of the nanosilver in Nanosilva. EPA has determined that the NOAEL is 49 $\mu g/m^3$ based on adverse histopathological patterns in lung tissue observed at the 117 $\mu g/m^3$ dose.

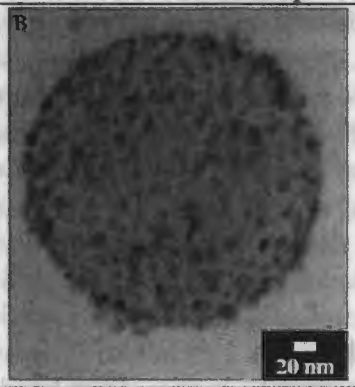
For short-term exposure, the target MOE of 1,000 is applied based on the standard 10-fold uncertainty factors for potential interspecies extrapolation and intraspecies variation, and a 10-fold database uncertainty factor. In addition, a 3-fold uncertainty factor (UF) was used to account for the lack of NOAEL in the 90 day oral toxicity study by Kim *et al.*, 2010 making the intermediate-term oral target MOE 3,000.

EPA does not typically consider long-term occupational exposures to antimicrobial preservatives used to treat plastics or textiles because application of these chemicals does not typically occur on a daily basis for more than 6 months. However, consumer exposure to Nanosilver may result in long-term exposure. The maximum recommended target MOE is 3,000 (U.S. EPA, 2002), which has already been established for intermediate-term exposures. Therefore, an additional UF for extrapolation from a subchronic study to chronic exposure durations has not been applied.

IV. STUDY WAIVER REQUESTS

1. Physical-Chemical Properties:

Table 1. Physical-Chemical Properties of Nanosilva.

Parameter	Nanosilva
Form	Liquid Suspension with 1% nanosilver by weight
Composition	Nanosilver-Silica Composite
Transmission Electron Microscopy (TEM) Image	
Overall Particle Size of Composite	Average of 320 nm
Shape of Nanosilver	Spherical
Size of Nanosilver (diameter)	3 to 18 nm
Coating	PVP and sulfur
Surface Charge	Negative

2. **Exposure scenarios:** EPA evaluates the risk of occupational exposures from mixing, loading, and applying or handling pesticide products. EPA expects that occupational inhalation and dermal exposures are likely to occur during mixing and loading of Nanosilva during preparation of a master batch. Nanosilva is proposed to be mixed into polymer and polymer-based products to suppress the growth of bacterial, algae, fungus, mold, and mildew, which cause odors, discoloration, stains, and deterioration of plastics and textiles. Because plastics incorporating Nanosilva could be subsequently used to manufacture children's toys and textiles worn by children, it is assumed that children will be exposed to products containing Nanosilva. EPA expects children's exposures are likely to occur during incidental oral and dermal exposures to plastics and textiles incorporating Nanosilva.

   To evaluate the risk from exposure to nanosilver that might break away from the Nanosilva complex, Nanosilva LLC submitted studies showing that silver at concentrations greater than the analytical detection limit were not found leaching from plastic coupons and shirts incorporating Nanosilva (Table 2). Thus, based on the available data, there is little exposure to nanosilver from plastic and textile incorporating Nanosilva. EPA calculated the daily-dose to workers assuming that all the silver in Nanosilva was freely available as nanosilver. Likewise for the daily-dose to consumers, EPA assumed that silver leaching from plastic coupons and shirts incorporating Nanosilva, which was at concentrations below the analytical detection limit, was in the form of nanosilver as found in Nanosilva.

Table 2. Amount of Silver Released from Shirts and Plastics Incorporating Nanosilva.

Parameter	**Nanosilva**	
Source of Information	Nanosilva Study	
Treatment Method	Incorporated into Shirts	Incorporated into Plastics
Amount of Silver Released (% of silver in article)	1.6 to 0.9%*	<0.1%*
Transmission Electron Microscopy (TEM) Image	Not enough silver to image	
Overall Particle Size of Composite	Not enough silver	
Shape of Nanosilver	Not enough silver	
Size of Nanosilver (diameter)	Not enough silver	
Coating	Not enough silver	
Surface Charge	Not enough silver	

* Calculated assuming that silver at a concentration of one-half the detection limit was released during leaching studies.

3. **Margins of Exposure (MOEs):** The MOE for occupational inhalation exposure to the nanosilver in the Nanosilva liquid suspension is 2,500 if workers use close-system loading when mixing and loading the Nanosilva liquid suspension (Target MOE=1,000). The MOE for dermal exposure to the nanosilver in the Nanosilva

liquid suspension is greater than 1,000,000 if workers use closed-system loading when mixing and loading the Nanosilva liquid suspension, which is significantly greater than the target MOE of 3,000. The MOEs calculated for the incidental oral exposure to nanosilver in plastic toys incorporating Nanosilva are 310,000 for children 6 to <12 months old, 550,000 for children 1 to <2 years old, and 940,000 for children 2 to <3 years old (Target MOE=3,000). The MOEs calculated for the dermal exposure to nanosilver in flooring incorporating Nanosilva are 820,000 for children 6 to <12 months old, 860,000 for children 1 to <2 years old, and 910,000 for children 2 to <3 years old (Target MOE=3,000). The MOEs calculated for the dermal exposure to nanosilver in textiles incorporating Nanosilva are 340,000 for children 6 to <12 months old, 360,000 for children 1 to <2 years old, and 370,000 for children 2 to <3 years old (Target MOE=3,000). The other route-specific scenarios for incidental oral exposure to flooring and textiles incorporating Nanosilva provide MOEs of 1,000,000 or higher.

The aggregate MOEs for children who are, within the same short time frame, mouthing a toy, flooring, and a textile containing Nanosilva while wearing a textile containing Nanosilva and crawling on flooring incorporating Nanosilva are greater than 100,000 for all lifestages, indicating that the risk for short- and intermediate-term simultaneous exposure to plastics and textiles incorporating Nanosilva is not a concern (Target MOE = 3,000; U.S. EPA, 2002). The calculated MOEs for dermal and incidental oral exposure to plastics and textiles incorporating Nanosilva are greater than 100,000, indicating that daily exposure to plastics and textiles incorporating Nanosilva for greater than 6 months are not likely to be of concern.

4. **Toxicity:** Nanosilva LLC submitted results from guideline acute animal-toxicity tests completed using high-level doses of a liquid suspension containing Nanosilva with 1% nanosilver by weight. There were no mortalities or abnormalities noted in test animals after administration of Nanosilva by oral, dermal, and inhalation routes at dose levels of up to 5,000 mg/kg and 2.05 mg/L, respectively. Nanosilva caused moderate to no irritation to skin or eyes at dose levels of up to 0.1 mL and 0.5 mL, respectively, and was not a skin sensitizer. The currently available subchronic oral toxicity studies indicate that nanosilver causes liver and kidney toxicity in laboratory animals where silver is distributed to all organs and tissues with accumulation of silver in the brain and male animal testes. Subchronic inhalation toxicity studies also identified liver toxicity as well as lung effects including chronic alveolar inflammation. There were potential neurotoxic effects identified with increases in neurotransmitter concentrations and loss of spatial cognition; however, these same effects were not observed in a follow-on study. The *in vitro* Ames and chromosome aberration assays indicate that nanosilver is not expected to be mutagenic while the mammalian cell micronucleus and mouse lymphoma with comet assay suggest that nanosilver may have mutagenic potential. However, the lack of genotoxicity from the *in vivo* study indicates that there is inadequate information to assess mutagenic (and hence carcinogenic) potential of nanosilver. Finally, there is not enough information on the reproductive and developmental toxicity for nanosilver at this time.

V. HASPOC WOE EVALUATIONS

1. **Inhalation Study:** Previously, the Office of Pesticide Programs (OPP) used a set of criteria to determine whether an inhalation study could be waived. These criteria considered the scientific information available for the chemical, including its: 1) degree of irritation and corrosivity; 2) volatility; 3) aerosol particle size; and 4) Acute Toxicity Category and extrapolated MOEs (e.g., MOEs 10 times higher than the target). In 2009, OPP developed an issue paper on risk assessment approaches for semi-volatile pesticides. As part of that issue paper, an analytical comparison was conducted of oral and inhalation experimental toxicology studies. In general, this analysis showed that the degree to which oral PODs were protective of potential inhalation toxicity varied. In many cases the oral POD was protective, but in some cases the inhalation PODs were significantly more sensitive. Currently, OPP uses a WOE approach that builds upon OPP's experience using the criteria listed above and conclusions from the 2009 SAP. As approaches for route-to-route extrapolation continue to evolve and improve, OPP may incorporate additional considerations into the WOE analysis.

   The HASPOC has concluded that a subchronic inhalation study with Nanosilva is needed to reduce the uncertainties related to the differences in the physical properties of the nanosilver particle used in the Song *et al.* (2012) study and the Nanosilva particles. This approach considered all of the available hazard and exposure information for nanosilver including: 1) the difference in the physical/chemical properties between the available nanosilver inhalation toxicity studies and the nanosilver in Nanosilva; 2) the sensitive effects noted in the inhalation study of decreases in physiological measures of lung function combined with chronic alveolar inflammation and macrophage accumulation in the lungs; and 3) the use of an inhalation POD which results in an MOE that is near the Agency's level of concern when exposure is assessed with a closed system.

2. **Subchronic Dermal Toxicity Study:** Based on a WOE approach, the HASPOC concludes that a dermal study is not required because the use of an oral POD results in MOEs that are ≥1,000,000 for occupational and greater than 300,000 for consumer exposure pathways. These MOEs are well above the Agency's level of concern.

3. **Prenatal Developmental Toxicity Study:** HASPOC has concluded that a reproduction/developmental toxicity screening test (Modified OCSPP 870.3550/ OECD TG 421) conducted via the inhalation route is needed. This determination is based on a WOE analysis which provides: 1) female workers of child bearing age will handle Nanosilva during mixing and loading; 2) as noted above, occupational MOEs are near the Agency's level of concern when exposure is assessed with closed system and additional information is needed to reduce uncertainty; 3) there are currently no acceptable studies on the reproductive and developmental toxicity for nanosilver. However, there were dose-dependent increases in the concentration of silver in the testes of rats after oral ingestion, inhalation, and injection of nanosilver and, in another study, nanosilver was distributed to major maternal organs and extra-

embryonic tissues although no adverse morphological effects on the developing embryos were observed.

4. **Bacterial Reverse Mutation Test, Detection of Gene Mutations in Somatic Cells in Culture, In Vitro Mammalian Cytogenetics, Mammalian Bone Marrow Chromosome Aberration Test**: The mutagenicity studies [Bacterial Reverse Mutation Test (OPPTS 870.5100), Detection of Gene Mutations in Somatic Cells in Culture (OPPTS *870.5300*), In Vitro Mammalian Cytogenetics (OPPTS 870.5375), Mammalian Bone Marrow Chromosome Aber*ration T*est (OPPTS 870.5385)] are satisfied because the available *in vitro* studies suggest that nanosilver may have mutagenic potential. The one available *in vivo* micronucleus assay performed on rats *after* oral administration of nanosilver at concentrations of up to 1,000 mg/kg/day (Kim *et al.*, 2008) indicated that nanosilver is neither *clastogen*ic nor aneugenic *in vivo*; however, there was no positive proof that nanosilver reached the bone marrow in the micronucleus assay. The HASPOC has concluded that an *in vivo* bone marrow assay is needed and that this assay can be added as a component of the subchronic inhalation study.

5. **Neurotoxicity Battery:** Acute and subchronic neurotoxicity studies can provide important scientific information on potential nervous system effects from pesticide exposure. These studies offer data on a wide range of functional tests for evaluating neurotoxicity including sensory effects, neuromuscular effects, learning and memory and histopathology of the nervous system. The study completed by Hadrup *et al.* (2012b), which reported significant increases in neurotransmitter concentrations (e.g., dopamine) after oral administration of nanosilver to rats at concentrations of up to 9 mg/kg/day, lacks histological support for determining NOAEL/LOAELs. Liu *et al.* (2013) reported no effects on the spatial cognition or hippocampal activity of mice after injecting nanosilver into the body cavity (i.e., intraperitoneal injection) at concentrations of up to 50 mg/kg. The effects on spatial cognition and hippocampal activity observed by Liu *et al.* (2012) after administering nasal drops containing nanosilver at concentrations of 3 and 30 mg/kg to rats suggest possible neurotoxic effects from the inhalation of nanosilver. However, EPA believes that the doses used in the Liu *et al.* (2012) study were greater than the maximum dose used in the inhalation toxicity study by Song *et al.* (2012). Unless appropriate study designs are used, many guideline studies are considered inadequate in their assessment of behavioral effects and do not use optimal methods to evaluate the potential toxicity to the nervous tissue structure and function. The HASPOC has concluded that there is potential for neurotoxicity based on the open literature studies. However, in lieu of a standard guideline study, neurotoxicity evaluation (functional observational battery, motor activity and detailed neuropathology) can be added as a component of the subchronic inhalation study.

6. **Immunotoxicity:** The HASPOC recommends that a waiver can be granted for an immunotoxicity study for Nanosilva, based on the following considerations:

- The currently available oral toxicity studies indicate that nanosilver causes liver and kidney toxicity in laboratory animals where silver is distributed to all organs and tissues with accumulation of silver in the brain and male animal testes. Inhalation toxicity studies also identified liver toxicity as well as lung effects including chronic alveolar inflammation.

- PODs from the most sensitive endpoints are currently used for assessing risks from short and intermediate oral and inhalation exposures.

- The toxicology database for nanosilver does not reveal any evidence of treatment-related effects on the immune system. The overall WOE suggests that this chemical does not directly target the immune system.

IV. HASPOC CONCLUSIONS

In summary, the HASPOC concludes that a subchronic inhalation study and a reproduction/developmental toxicity screening study are needed. In addition, data to evaluate the potential for neurotoxicity and *in vivo* bone marrow assay are needed. Immunotoxicity and subchronic dermal studies are not needed and can be waived.

The HASPOC recommends that the registrant submit protocols for the needed studies for the Agency to review. If the registrant has additional exposure or toxicity information which could be used to further refine the data needs described here for Nanosilva, the registrant may submit a rebuttal for review.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

April 12, 2013

NanoSilva, LLC
2811 NE 14th Street
Ocala, FL, 34470

Attention: Wayne Krauss

Subject: Review of Results from Studies to Determine the Amount and Size Distribution of Silver Released from Textiles Treated with Nanosilva (NSPW-L30)

In August, 2009 Nanosilva LLC submitted an application to register Nanosilva as a new active ingredient under FIFRA section 3(c)(5). Nanosilva LLC submitted acute toxicity studies required under 40 CFR 161 and a study that determined the percentage of silver released from Nanosilva treated plastic coupons. In July, 2012, EPA notified Nanosilva LLC that before EPA can evaluate the risk from exposure to Nanosilva treated textiles, information on the amount and size distribution of silver that consumers, workers, and the environment are exposed to from wearing, manufacturing, and washing textiles treated with NanoSilva is necessary.

Nanosilva LLC agreed to conduct additional studies required to support the use of Nanosilva as a materials preservative in textiles. Nanosilva LLC prepared and submitted on August 20, 2012 a draft protocol titled "The Quantification and Characterization of Silver Released from Textiles Treated with NanoSilva (NSPW-L30) as a Results of Washing" with the stated purpose "to quantify and characterize silver possibly released from textiles treated with NSPW-L30 as a result of laundering." On September 28, 2012, EPA provided recommendations to improve the protocol.

On March 8, 2013 you submitted the following two study reports concerning the release of silver from textiles treated with Nanosilva:

1. The Quantification and Characterization of Silver Released from Textiles Treated with NanoSilva (NSPW-L30) as a Result of Washing
2. The Quantification of Silver Released from Textiles Treated with NSPW-L30SS as a Result of Simulated Contact/Exposure Conditions with Synthetic Saliva

We have reviewed these two studies and concluded that major revisions to the two study reports are required before the study results can be used in reaching a decision regarding your application to register Nanosilva for use as a textile preservative. The enclosures to this letter contain detailed comments for each of the two study reports, what follows is a summary of the major deficiencies that need to be addressed.

1. There is no documentation that samples fortified with 0.012 ug/L silver were prepared or analyzed. It appears, on Page 36 of Study 1 and Page 34 of Study 2, that the method detection level (MDL) was determined using laboratory reagent blanks instead of samples fortified with 0.012 µg/L of silver. Thus, documentation to establish the level of quantitation has not been presented for either study leading to uncertainty in the stated level of quantitation (LOQ) of 0.094 µg/L for liquids and 9.4 µg/kg for solids.

2. Preparation, use, digestion, and analysis of the 0.45 µm filters are unclear.

- Were these filters cut to fit into a housing?
- What is the make, model, and diameter of the filter apparatus?
- Why were the filters cut into small pieces after being used to filter the wash and rinse solutions? Were any experiments performed to determine if there was a loss of silver from cutting the filters?
- The concentration of silver in the filters is reported as "ppb": there is no documentation demonstrating whether this concentration has units of µg/kg or µg/L. Without this documentation, there is no way to calculate the mass of silver on the filters or the percentage of silver released from Nanosilva treated textiles.
- The term "filter/residue" is very confusing for all reviewers. What is meant by the term "residue"?

3. There was no discussion of the quality assurance sample results. You will need to report and discuss results for:

- ICV and CCV samples
- The 10 mg/L silver textile spike
- The 5 mg/L silver filtration spike

4. There was no discussion of control sample results. What do the results from the white untreated textile tell us about the concentration of silver found in the filtrate and filter from the Nanosilva treated textiles? Where is the discussion on the results from the unused filter paper samples?

5. Use of the term "recovery rate" is not appropriate.

- Effective silver yield should be used in the section on Theoretical Silver Content of Treated Textile.
- Mass of silver lost during washing is based on the difference between the amount of silver in textiles before and after washing.
- Mass of silver recovered from washing is based on the concentration of silver detected in the filtrate and filters.

6. The concentrations and amounts of silver are reported with as many as ten significant figures. These values should be reported with the appropriate number of significant figures, which in most cases, will be two or three.

7. The concentrations of silver are listed by sample number and sample ID on the Test Reports, sample number and flask number on the Heavy Metal Analysis Worksheets and by flask number (listed as sample name) on the Quantitation Reports. It would be helpful if these silver concentrations could be consolidated into one table with columns for sample ID, sample number and flask number.

8. There is no way to verify concentration values from raw ICP-MS CPS counts. There is no information about which calibration curve was used and the volume or mass of sample employed to calculate the concentration of silver in liquid and solid samples. Ideally, reviewers should be able to use the raw ICP-MS CPS counts and independently calculate the concentration of silver found in each sample.

9. There are many typographical errors and miscalculations in this document. Great care must be taken to revise these documents so that scientific reviewers can easily find all information and comprehend the discussion and conclusions as well as verify reported concentrations. For example, the percentage of silver released from washing the Nanosilva treated textile is the value EPA needs to calculate the risk to children who wear and chew on Nanosilva treated textiles and to the environment which receives the water used to wash the Nanosilva treated textiles. Although this value is reported in Table 10 of Study 1 as 0.353% and in Table 11 of Study 2 as 0.102%, we have not been able to verify these values through independent calculation.

10. Why are there two separate study results when much of the General Information, Materials, and Methods are largely the same for both studies?

A decision regarding your application to register Nanosilva for use as a textile preservative cannot take into account the results from the leaching studies until the above deficiencies have been addressed. The leaching study reports, in current form, are not acceptable for evaluating the use of Nanosilva in textiles. Scientific report format

requires that all results be analyzed, be clearly presented so readers can verify the conclusions presented, and that calculated values can be verified using the raw analytical results. Without this level of detail and clarity, reviewers question the reliability of test results. Because EPA makes science based decisions, we can't rely on results where significant questions of reliability exist.

In the absence of leaching data that is free of deficiencies, the Agency will use the standard assumptions that 100% of Nanosilva in treated textiles is transferred to the skin of people who wear these textiles and that 50% of the Nanosilva in the treated textile is ingested by children who chew on these textiles. Given that use of these standard assumptions is likely to result in a risk concern for use of Nanosilva in textiles, it would be advisable for Nanosilva LLC to address the leaching study deficiencies. EPA may accept these studies depending on the outcome of your efforts to correct the deficiencies cited above and in the detailed comments provided in the enclosures.

On March 13, 2013 you requested that the PRIA date be revised from March 29, 2013 to July 31, 2013. To meet this PRIA date, EPA will have to post the document regarding the decision to register Nanosilva for public comment by June 3, 2013. Unless Nanosilva LLC can provide revised leaching studies that address the concerns discussed herein by April 29, 2013, EPA will not have sufficient time to incorporate these study results into the decision document prior to posting for public comment.

Sincerely,

Jed Costanza, Ph.D.
Regulatory Management Branch 1
Antimicrobials Division (7510P)

Enclosures (2)
1. EPA Comments on Nanosilva Detergent Leaching Study dated April 10, 2013
2. EPA Comments on Nanosilva Saliva Leaching Study dated April 10, 2013

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

September 28, 2012

NanoSilva, LLC
2811 NE 14th Street
Ocala, FL, 34470

Attention: Wayne Krauss

Subject: Recommendations to the Protocol for Determining the Amount and Size Distribution of Silver Released from Textiles Treated with NanoSilva (NSPW-L30)

In your email of July 27, 2012, NanoSilva LLC agreed to conduct additional studies required to support the use of NanoSilva as a materials preservative in textiles. You stated that NanoSilva will be incorporated into polymer fibers before being woven into non-organic blend textiles and that NanoSilva will not be applied during post-production textile treatment. Before EPA can evaluate the risk from exposure to NanoSilva treated textiles, information on the amount and size distribution of silver that consumers, workers, and the environment are exposed to from wearing, manufacturing, and washing textiles treated with NanoSilva is necessary.

In a July 31, 2012 email, I recommended that you consult the following documents prior to preparing a protocol to determine the amount and size distribution of silver released from NanoSilva treated textiles:

1. Geranio, L., Heuberger, M., Nowack, B. 2009. The Behavior of Silver Nanoparticles during Washing. *Environmental Science and Technology* 43:8113-8118.
2. Lorenz, C., Windler, L., von Goetz, N. et al. 2012. Characterization of silver release from commercially available functional (nano)textiles. *Chemosphere* 89:817-824.
3. ISO Colour Fastness Test. Textile test for Colour Fastness part C06: Colour Fastness to Domestic and Commercial Laundering, ISO 105-C06; International Organization for Standardization (ISO): Geneva, 1997.

The studies by Geranio et al. (2009) and Lorenz et al. (2012) involved washing textiles treated with silver and nanosilver to determine the amount and form of silver released to

the environment. These studies used a modified version of the ISO Colour Fastness test as the basis for their textile washing method.

The ISO Colour Fastness test is thought to represent aggressive washing conditions with one wash cycle representing up to five domestic or commercial laundering cycles when the multiple test is employed. The amount of silver released during one ISO Colour Fastness test is believed to exceed the actual daily dose of nanosilver from a treated textile because the ISO Colour Fastness test involves immersing the textile in water containing detergents and steel balls followed by mechanical agitation for 45 minutes. Thus, results from studies which are based on the ISO Colour Fastness test will be used to determine the daily dose of nanosilver for children who chew and mouth, adults who wear, and workers who manufacture items from nanosilver treated textiles even though this likely overestimates the daily dose of nanosilver.

In response to EPA recommendations, NanoSilva LLC prepared and submitted on August 20, 2012 a draft protocol titled "The Quantification and Characterization of Silver Released from Textiles Treated with NanoSilva (NSPW-L30) as a Results of Washing" with the stated purpose "to quantify and characterize silver possibly released from textiles treated with NSPW-L30 as a result of laundering." Nanosilva LLC used the study by Lorenz et al. (2012) along with the ISO Colour Fastness test as the basis of the protocol for testing NanoSilva treated textiles.

Your draft protocol proposes to determine the:

- Initial silver content of three NanoSilva treated textiles
- Amount and form of silver released from samples of each of three NanoSilva treated textiles using a modified version of the ISO Colour Fastness test
- Silver content of each sample after washing

Completing these steps will allow you to demonstrate that the amount of silver released during washing NanoSilva treated textiles is consistent with the amount of silver missing from the textile.

The proposal states that the amount and form of silver released from NanoSilva treated textiles will be determined in the following phases:

- Phase One: determine the silver content of three treated textiles
- Phase Two: washing of one sample from each treated textile
- Phase Three: analysis of the washing and rinsing solutions for silver content
- Phase Four: determine the silver content of the washed textile
- Phase Five: calculate the silver recovery rate

- Phase Six: if silver is detected; SEM will be utilized to determine the species of silver detected in the washing and rinsing solutions.

Recommendations

The following sections provide EPA recommendations on the protocol. In summary the EPA recommends:

1. Improve the description for the NanoSilva treatment and nanosilver content of textiles
2. Wash each sample of NanoSilva treated textile in a separate vessel
3. Increase the NanoSilva treated textile sample size to 8 cm by 20 cm
4. Perform a wash test with simulated human saliva in addition to the tap water with detergent wash
5. Use STEM instead of the SEM in examining silver released during washing
6. Use a 3 kDa ultrafiltration membrane to separate ionic silver from nanosilver in the 0.45 μm filtrate
7. Employ a silver nitrate solution to quantify the loss of ionic silver during silver fractioning

Treated Textile: Please provide a description of how textiles are treated and please spell out PBT. For example, in your July 27, 2012 email you stated:

> "…which incorporate polymer based fibers integrated (extruded into the fiber during the fiber production process) with NanoSilva. The Fabric use would only be applicable to polymer based Fibers (i.e. polyester, nylon, P.P. etc.) and would be limited to use in only non-organic blends. This is not a post-production treatment use (coating or dipping)."

EPA believes that you are incorporating NanoSilva into polymer based fibers that are to be woven into textile products, please provide more details on this process.

Please expand this section so that the amount of nanosilver in NanoSilva (NSPW-L30) and treated textiles is clear. EPA believes that a 10% master batch concentration would yield a nanosilver concentration of 20 ppm in the final treated article according to the following calculations:

Amount of nanosilver in the master batch:

$$\frac{0.01\ g\ nanosilver}{g\ Nanosilva} \times \frac{0.02\ g\ Nanosilva}{g\ master\ batch} = \frac{0.0002\ g\ nanosilver}{g\ master\ batch}$$

Maximum amount of nanosilver in the treated article:

$$\frac{0.0002\ g\ nanosilver}{g\ master\ batch} \times \frac{0.10\ g\ master\ batch}{g\ treated\ article} = \frac{0.00002\ g\ nanosilver}{g\ treated\ article}$$

$$\frac{0.00002\ g\ nanosilver}{g\ treated\ article} \times 100\% = 0.002\%\ \frac{nanosilver}{treated\ article}$$

$$\frac{0.00002\ g\ nanosilver}{g\ treated\ article} \times \frac{1000\ mg}{g} \times \frac{1000\ g}{kg} = 20\ ppm\ \frac{nanosilver}{treated\ article}$$

In the background section the statement is made: "The proposed application rate in the final treated article is 10-30 ppm of silver or 5-15 percent Master Batch." Is NanoSilva seeking to increase the application rate to 30 ppm? If this is the case then we recommend testing textiles containing 30 ppm of nanosilver.

Washing of Treated Textiles: The protocol proposes to use 150 mL of washing solution to which 10 rubber balls are added to evaluate the release of silver from 4 cm by 10 cm samples of NanoSilva treated textile after a 45 minute wash cycle at 40 degrees Celsius. These conditions are consistent with the multiple test as described in the ISO Colour Fastness test where one wash cycle represents up to five domestic or commercial laundering cycles.

The protocol proposes to include two 5 min rinse cycles to recover all the silver released after the 45 min wash cycle. Although EPA would prefer that the 5 min rinse cycle sample be analyzed separately from the 45 min wash cycle sample, these may be combined to save on analysis costs since EPA will used the overall amount of silver released from both the 45 min wash cycle and the 5 min rinse cycle in calculating the daily dose of nanosilver for NanoSilva treated textiles.

EPA recommends that each sample of NanoSilva treated textile be washed in a separate vessel, so that there is one textile sample per washing vessel.

EPA notes that the ISO Colour Fastness test calls for use of a 4 cm by 10 cm section of textile sewn to the dyed textile to evaluate staining resulting from desorption of textile dyes. Although fabric staining is not a concern for NanoSilva treated textiles, it is recommended that the NanoSilva treated textile sample size be increased to 8 cm by 20 cm to maintain the same washing solution to textile area ratio specified in the ISO Colour Fastness test.

Although the ISO Colour Fastness test stipulates the use of detergents with tap water as the wash liquid, because EPA must also evaluate the release of nanosilver when children chew and mouth NanoSilva treated textiles, EPA recommends including an additional test using simulated human saliva. EPA recommends using as wash liquid prepared according to the recipe for the "SAGF" medium as found in:

Gal, J.Y., Fovet, Y., Abid-Yadzi, M. 2001. About a synthetic saliva for in vitro studies. Talanta 53:1103-1115.

EPA must evaluate the risk of dermal exposure to nanosilver for children who wear NanoSilva treated textiles. EPA does not anticipate dermal exposure will result in a risk concern for NanoSilva treated textiles and therefore, a separate leaching study involving simulated human sweat is not recommended. EPA will use the results of the ISO Colour Fastness test completed with detergents and tap water to evaluate the dermal exposure to nanosilver from NanoSilva treated textiles.

Analytical: The analytical methods proposed include:

- ICP-OES to determine the amount of silver in the NanoSilva treated textiles
- ICP-MS to determine the amount of silver released after washing NanoSilva treated textiles
- SEM/EDX to determine the form of silver in the wash and rinse water

The ICP-OES and -MS instruments are both suitable for determining the concentration of silver, however, it is not clear why both of these instruments are being employed. We recommend choosing one of these two ICP based instruments to minimize calibration efforts and simplify the interpretation of analytical results. SEM with EDX is suitable for determining the shape, identity, and size distribution of silver. This technique will not provide information on the oxidation state of silver.

Although the instruments you propose are acceptable, we recommend you consider the following additional instrumentation:

1. Scanning transmission electron microscopy (STEM) may be a better choice because silver is known to undergo charging during SEM which may distort its image.
2. UV-Visible spectroscopy is a convenient way to scan suspensions for presence of nanosilver as indicated by a surface plasmon resonance peak at approximately 400 nm wavelength.

If microscopy is used to determine the size distribution of silver released after washing NanoSilva treated textiles, EPA recommends consulting the following International Standards Organization standard:

ISO 13322-1:2004 Particle size analysis—Image analysis methods—Part 1: Static image analysis methods

If you chose to determine the size distribution using microscopy and don't employ the methods described in ISO 13322-1:2004, then you run the risk of EPA finding that your particle size distribution data are unacceptable.

Silver Fractioning: The protocol proposes to determine the form of silver released after washing NanoSilva treated textiles by passing the wash water through 0.45 µm pore size filters. The filters which will theoretically retain particles with diameters greater than 0.45 µm or 450 nm will be digested and analyzed for silver content. The wash water that passes through the 0.45 µm pore size filters will theoretically contain particles having diameters of less than 0.45 µm or 450 nm along with ionic silver. EPA recommends that the water which passes through the 0.45 µm filter be further filtered through a 3 kDa ultrafiltration membrane. The secondary filtration will separate the particulate silver from ionic silver and allow NanoSilva LLC to distinguish between nanosilver and ionic silver. EPA also recommends that filtration of a standard silver nitrate solution be employed to quantify the amount ionic silver that is lost during the 0.45 µm and 3 kDa filtration steps.

Calculation of ecovery: Recovery of silver is proposed to be calculated by comparing the initial silver content of the NanoSilva treated textile with the silver content of the textile after washing. EPA recommends including the amount of silver recovered in the wash water as part of the silver recovery calculation.

In closing, the preceding recommendations are provided to improve the protocol that NanoSilva LLC plans to use for determining the amount and size distribution of silver that consumers, workers, and the environment are exposed to from wearing, manufacturing, and washing textiles treated with NanoSilva. This review does not constitute acceptance or approval of the results generated by using this protocol.

In a July 30, 2012 email to you, EPA estimated that if NanoSilva LLC provides the results of studies determining the amount and size distribution of silver released from NanoSilva treated textiles by November 30, 2012, EPA will review this data and prepare a decision document for the proposed conditional registration of NanoSilva as a materials preservative in plastics and textiles by December 28, 2012. EPA will post this decision document for a 30 day public comment period by January 31, 2013 and endeavor to make a decision regarding the conditional registration of NanoSilva as a materials preservative in plastics and textiles by the PRIA date of March 29, 2013. If NanoSilva LLC fails to submit the leaching study results by November 30, 2012, EPA may not have enough information to make a registration decision on your application by the PRIA date of March 29, 2013.

Sincerely,

Jed Costanza, Ph.D.
Regulatory Management Branch 1
Antimicrobials Division (7510P)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES

Jan.09, 2012

MEMORANDUM

SUBJECT: Review of: Determination of Silver Content and Silver Recovery Rate for NSPW-L30SS

PC Code(s): 072501	DP Barcode(s)/No(s): 397520 PRIA
Decision No.: 418580	Reregistration No(s).
Petition No(s).: NA	Regulatory Action: Availability of (nano) silver through migration from treated article): product registration
Risk Assess type:	Case No(s):
TXR No.: NA	CAS No(s): 7440-22-4
MRID No(s).: 486529-01	40 CFR: NA

From: A. Najm Shamim, PhD, Chemist
Risk Assessment & Science Support Branch
Antimicrobials Division (7510P)

Thru: Nader Elkassabany, PhD, Chief
Risk Assessment & Science Support Branch
Antimicrobials Division (7510P)

To: Dennis Edwards, Chief
Regulatory Management Branch
Antimicrobials Division (7510P)
&
Jed Costanza, PhD, Environmental Engineer
Regulatory Management Branch 1
Antimicrobials Division (7510P)

Background:

Nanosilva conducted a Migration (Leaching) study from Food Contact Substance. It was a non-guideline study (Nanosilva, 2009). The Agency reviewed the study and did not find acceptable. A number of deficiencies were noted by the Agency and pointed out to the registrants. Specifically the following deficiencies were critical in the outcome of the study:

1) The actual concentrations of silver and Nanosilva in the plastic test polymers is unclear. The beginning of the report states that the three concentrations of the colloid in the test polymers are 0.00125%, 0.0025% and 0.005%. However, based on the manufacturing information in Addendum 7 of the study report, the level of Nanosilva in the three types of coupon would be 0.125%, 0.25% and 0.5%. Finally, the experimentally determined concentrations of the colloid in the three polymers tested (based on silver content) was 0.005%, 0.034%, and 0.059%.

2) In the procedures describing the production of the "master batch", it is unclear exactly how much Nanosilva colloid was added to the plastic resin.

3) Based on the experimental determinations of their silver content, the colloid concentration of the "2.5%" plastic coupons was more than 3 xs below the target concentration.

The Agency further said that: "The Agency recommends that the registrants submit new protocols to run a new study, get the protocols approved by the Agency before conducting the study. The protocols can be developed based on removing the deficiencies the Agency has noted in the present review. The analytical methods to be used for the new study should be able to identify the characteristics of nanocomposite, silver that is being leached out and other moieties found during the study.
In addition, we recommend that the registrants must take into account the deficiencies noted in the DER on Product Chemistry data of Nanosilva (Memo by Earl Goad)"
(AD Memo from: A. Najm Shamim to Demson Fuller, CRM for Nanosilva, June 28, 2010)
In this regard as a follow up:
1)Jed Costanza communicated with to Nanosilva regarding the reasons of the unacceptability of the study (Dec. 15, 2010).
2)On Dec. 17, 2010 Nanosilva provided revised estimates of theoretical silver contents of the coupons.
3) On Dec. 20, AD courtesy Jed Costanza forwarded a Memo to Nanosilva outlining AD recommendations for silver content determinations in the plastic coupons using various analytical techniques.
4) On March 2011, Nanosilva informed AD that Nanosilva will determine the silver content in new plastic coupons, and also would complete a new leaching study to determine silver to replace the old leaching study.

To this end Nanosilva completed a new study on the silver content and silver recovery rate for their product (to be registered) NSPW-L30SS in its technical (which is colloidal) state and is compounded (Master Batch), and incorporated into a treated article (Coupon, End-use Product)
Note: The Agency decided it would accept the 60% as the minimum recovery level for the new study.

The study reviewed in this Memo was submitted on Nov.8, 2011. With this submission Nanosilva also informed AD that they will NOT be able to conduct the leaching study as previously suggested by them as enough funding is not available at this time.

Introduction

The present study was divided into three phases:

1. Phase one: Analysis of NSPW-L-30SS (colloid): This was done to calculate the theoretical silver content, and calculation of silver recovery for the test samples. The analytical technique used was Neutron Activation Analysis (NAA).
2. Phase two: Analysis of Master Batch (2% of NSPW-L30-SS). Same process was followed in this phase as in phase one for the Master Batch Analysis.
3. Phase three: Analysis for Coupon (containing 5% Master Batch): Same methodology was used in this phase of the study as in phases one and two.

Discussion:

The major cause for the rejection of previous study was the contractions in calculations, and subsequently the results obtained from these calculations were erroneous. All three phases of the study were designed in such a way the results of the first phase were used for phase two and the results from phase two were used for phase three, to arrive at consistency in results.

For phase one: Analysis was done on the colloidal (yellow) solution which had theoretical silver concentration of 1.19% (by wt.) Thus theoretical silver content was: 11,908 ppm. Three such samples were subjected to Neutron Activation Analysis (NAA). Three aliquots of each sample were analyzed (hence n =9 samples in all).
The percent recovery of the measured silver content ranged between 66.69 to 88.58 and the mean value was at 77.40% or 9216.7 ppm vs. 11,908 ppm (theoretical).
The remaining samples were returned to the registrants to produce Master Batch and coupons for phases two and three study.
The three samples of the colloids were:
1) 003-001-110215, and this one gave the average measured value of 8094.1 ppm of silver (vs. theoretical of 11908 ppm);
2) 003-002-110215 gave an average measured value of 9552.o (vs. 11908 ppm theoretical value) and
3) 003-003-110215 gave the average measured value of 10004.2 ppm of silver (vs. theoretical value of 11908 ppm), the average of the three averages was: 9216.7 ppm as noted above.

For phase two: 80 g of the colloids of the phase one solution was dissolved in 3920 g of low density polyethylene (LLDPE) to create a 2% composite (Master Batch):

Taking into account for 003-001-110215 the theoretical content to be 8094 ppm, in 2% composite the theoretical content of silver is 162 ppm, and similarly
for 002 and 003 samples the theoretical silver content is 191 and 200 ppm respectively.

These Master Batch samples were subjected to NAA, and the results obtained were:
Sample 001: 169 ppm of silver (vs. 162 ppm theoretical);
002 sample: 181 vs. 191 ppm of silver and
Sample 003: 175 vs. 200 ppm of silver
Percent recovery averaged: 95.11 and the average value of 175 ppm vs. 184 ppm of silver (theoretical value).

Phase three (Analysis of the Coupons): 20 g of the phase two (Master Batch) samples were taken and mixed with 380 grams of LLDPE for three minutes;(thus 5% samples were created) the mixture of the two was injected into the standard coupons through Boy 155 Injection-Molding Machine at a temperature of 180° C. Five coupons for each Master Batch samples were made but each coupon was cut into six chips and packaged (each packaged bag contained 30 chips).
So sample 001 from Master Batch (measured value of 169 ppm) contained 169 x .05 = 8.45 ppm of silver, and similarly the other Master Batches contained 9.05 and 8.75 ppm of silver respectively.
The silver content of these coupons (chips) were analyzed through NAA by random selection of chips from each bag of chips (sample 001 bag, 002 bags etc.)
For the samples containing 8.5 ppm, 9.05, and 8.75 ppm silver, the average measured value came out to be 8.7 ppm of silver. (Per cent recovery 99%)

RASSB Conclusions:

1) The study is acceptable
2) The low silver percent recovery in the colloidal solution (phase one) may be due to many reasons: silver loss due to the colloidal formation, silver becomes entrapped in the complex matrix or complexation of some silver with the sulfur or oxygen in the matrix, and this could reduce the 'available silver' for chemical analysis or reactivity of the total silver present.
3) It must be pointed out that the previous study on migration of silver from the coupons showed consistent and reasonable results at pHs 2, and 8.

BIBLIOGRAPHY

1. An AD MEMO From A. Najm Shamim to Demson Fuller (June , 2010)
2. An AD Product Chemistry Review of NanoSilva by Earl Goad, July 2010

October 26, 2011

Mr. Marshall Swindell
Product Manager 33
One Potomac Yard
2777 Crystal Drive
Arlington, VA. 22202

Subject: Non-Guideline Study Submittal for New Chemical Registration (A420 PRIA)

Dear Marshall,

NanoSilva LLC has completed the following Non-Guideline Study, Determination of Silver Content and Silver Recovery Rate for NSPW-L30SS, in response to the U.S. Environmental Protection Agency request.

Thank you for your continued support.

Regards,
NanoSilva LLC

Wayne Krause
V.P. Operations

TRANSMITTAL DOCUMENT

Name and Address of Submitter:	Nanosilva, LLC 2811 NE 14th Street Ocala, Florida 34470
Regulatory action in support of which this package is submitted:	**Application for Registration of Nanosilva™ Antimicrobial [Silver – Silica Colloid – (NSPW-L30SS – Product Code)]**
EPA Reg. No./File Symbol:	No. 84610
Alternate Test Material Names:	Covalently bonded Silver-Silica Colloid in aqueous solution.
Transmittal Date:	10/26/2011

Administrative Materials

Transmittal Document
Cover Letter

Volume No.	Citation	MRID Number
	Determination of Silver Content and Silver Recovery Rate for NSPW-L30SS; Non-Guideline Study, Wayne Krause, 2011 Unpublished study by NanoSilva, LLC. 24 pages.	**48652901**

Company Official: Wayne Krause, Vice Pres. Operations
Company Name: Nanosilva, LLC
Company Contact: Wayne Krause, (770) 687-8743 , fax: (352)-368-1796, wkrause@clairson.com

NANOSILVA, LLC.

February 3, 2011

84610-E

Mr. Marshall Swindell
Product Manager 33
One Potomac Yard
2777 crystal Drive
Arlington, VA. 22202

Subject: Supplemental Submission for New Chemical Registration (A420 PRIA)

Dear Marshall,

Nanosilva, LLC has prepared the following supplemental submissions in response to the U.S. Environmental Protection Agency request:

- Confidential Statement of Formula EPA Form 8570-4
- Supplement to MRID 47828903
  - Facile route for preparation of silica-silver heterogeneous nanocomposite particles using alcohol reduction method.
  - Preparation of silica-silver heterogeneous nanocomposite particles by One-pot preparation strategy using polyol process: Size controlled immobilization of silver nanoparticles.
- Supplement to MRID 47828904
  - Facile route for preparation of silica-silver heterogeneous nanocomposite particles using alcohol reduction method.
  - Preparation of silica-silver heterogeneous nanocomposite particles by One-pot preparation strategy using polyol process: Size controlled immobilization of silver nanoparticles.

In closing, we would like to thank you for your time and consideration during this registration process. Additional submissions are forthcoming.

Regards,
NanoSilva, LLC.

Wayne Krause
V.P. Operations

TRANSMITTAL DOCUMENT

Name and Address of Submitter:	Nanosilva, LLC 2811 NE 14th Street Ocala, Florida 34470
Regulatory action in support of which this package is submitted:	**Application for Registration of Nanosilva™ Antimicrobial [Silver – Silica Colloid – (NSPW-L30SS – Product Code)]**
EPA Reg. No./File Symbol:	No. 84610
Alternate Test Material Names:	Covalently bonded Silver-Silica Colloid in aqueous solution.
Transmittal Date:	02/02/2011

Administrative Materials	
	Transmittal Document
	Cover Letter
EPA Form 8570-4	Confidential Statement of Formula (3 copies)

Volume No.	Citation	MRID Number
	Supplemental Submission to MRID 47828903 (Description of Production Process), Published study by Jong-Min Lee, Dae-Wook Kim, Young-Doo Jun, Seong-Geun Oh, 2006 Preparation of silica-silver heterogeneous nanocomposite particles by One-pot preparation strategy using polyol process: Size controlled immobilization of silver nanoparticles.	**48379901**
	Supplemental Submission to MRID 47828903 (Description of Production Process), Published study by Jong-Min Lee, Dae-Wook Kim, Young-Doo Jun, Seong-Geun Oh, 2006, Facile route for preparation of silica-silver heterogeneous nanocomposite particles using alcohol reduction method.	**48379902**
	Supplemental Submission to MRID 47828904 (Description of Formulation Process), Published study by Jong-Min Lee, Dae-Wook Kim, Young-Doo Jun, Seong-Geun Oh, 2006 Preparation of silica-silver heterogeneous nanocomposite particles by One-pot preparation strategy using polyol process: Size controlled immobilization of silver nanoparticles.	**48379903**
	Supplemental Submission to MRID 47828904 (Description of Formulation Process), Published study by Jong-Min Lee, Dae-Wook Kim, Young-Doo Jun, Seong-Geun Oh, 2006, Facile route for preparation of silica-silver heterogeneous nanocomposite particles using alcohol reduction method.	**48379904**

Company Official: Wayne Krause, Vice Pres. Operations
Company Name: Nanosilva, LLC
Company Contact: Wayne Krause, (352)-615-4906 , fax: (352)-368-1796, wkrause@claiı son.com

DF

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

Nanosilva, LLC
2811 NE 14th Street
Ocala, Florida 34470

SEP 2 8 2010

Attention: Mr. Wayne Krause

Subject: **Nanosilva TM Antimicrobial**
- Product Chemistry, Acute Toxicity, and Migration Studies
EPA Reg. No. 84610-E

Dear Mr. Krause,

We have completed reviews of the product chemistry, acute toxicity, and migration studies that were submitted in support of your application for registering the Nanosilva™ Antimicrobial additive. The following sections summarize our findings of these reviews. Copies of our reviews are enclosed. Please refer to the reviews for further details on the chemistry studies that are not acceptable and the concerns identified with the leaching study. The deficiencies will need to be addressed.

Product Chemistry:

The following data requirements were acceptable as submitted:
830.1600, 830.1750(g), 830.6302, 830.6303, 830.6304, 830.6319, 830.6320, 830.7000, 830.7100, 830.7220, and 830.7300.

The following data requirements were not acceptable as submitted:
830.1550, 830.1620, 830.1650, 830.1670, 830.1750, 830.1800, and 830.6317.

Acute Toxicity:

The following data requirements were acceptable as submitted:
870.1100, 870.1200, 870.1300, 870.2400, 870.2500, and 870.2600

Migration (Leaching) from Food Contact Substance:

The submitted non-guideline study on the leaching of silver from an LLDPE polymer containing various concentrations of the Nanosilva™ Antimicrobial additive is not

acceptable as submitted. Given that the recoveries of silver from the fortified leachates with control coupons were less than 70%, except for the studies completed in the pH 2.0 and pH 8.0 water, and the 10% NaH2PO4 solution, the migration values might be undervalued.

We have not made a decision on whether the polymer leaching study will support all of the uses that you have proposed for this product. We will inform you in the near future whether additional leaching studies are needed and if so, which use patterns will require such a study.

Once you have read the reviews, we will be glad to schedule a meeting or conference call to discuss the additional chemistry information needed and to discuss our review of your leaching study. In fact, I would encourage you to discuss with us how you will address the needed information before conducting the studies to ensure that we get the information that we need.

If you have any questions concerning this letter, please contact me at 703-308-6341.

Sincerely,

Marshall Swindell
Product Manager 33
Antimicrobials Division (7510P)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES

Wovin cmo / covers all uses

06/28/2010

MEMORANDUM

DATA EVALUATION RECORD

SUBJECT: Study: Migration (Leaching) from Food Contact Substance
(Non-guideline)

From: A. Najm Shamim, PhD, Chemist
Risk Assessment & Science Support Branch
Antimicrobials Division (7510P)

To: Demson Fuller, CRM for NanoSilva
Regulatory Management Branch 1
Antimicrobials Division (7510P)

Thru: Nader Elkassabany, PhD., Chief
Risk Assessment & Science Support Branch
Antimicrobials Division (7510P)

Note: The original review was done by: Pesticides Health Effects Group,
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713
As a Subcontractor to: ICF International
9300 Lee Highway
Fairfax, VA 22031

PC Code: 072501
MRID #: 47828925
DP Barcode: (370735)

TEST MATERIAL (PURITY): Nanosliva™ Antimicrobial (~40.4% nano-silver-silica particles; 1.19% silver)

SYNONYMS: Silver-Sulfur-Silica Complex; Sliver-Silica colloid; NSPW-L30SS

CITATION: Kmieck, PJ (2009). Leaching Protocol for Nanosilva Antimicrobial Treated LLDPE Polymer in Food and Food Simulated Matrices as functions of Time Temperature and Chemistry of the Matrix with Determined Migration Values.

Study Number 090106-1754-55-74. April 24, 2009.

MRID 47828925. Unpublished.

SPONSOR: Nanosilva Antimicrobial, LLC, 2811 NE 14th Street, Ocala, FL, USA

COMPLIANCE:

Signed and dated Good Laboratory Practices (GLP), Quality Assurance and Data Confidentiality statements were provided.

EXECUTIVE SUMMARY: A non-guideline study (MRID 47828925) was conducted investigating the leaching/migration of nano-scaled silver embedded into a low-density polyethylene (LLDPE) plastic polymer as a nano-silver-silica particles composite (as Nanosilva colloid or NanoSilva™ Antimicrobial colloid, and an attempt was made to estimate the amounts of leachates (silver) into a variety of food simulants. The Nanosilva colloid was incorporated into the LLDPE polymer at reported concentrations of 0.00125%, 0.0025% and 0.005%; however, experimental concentrations of Nanosilva were determined to be at 0.005%, 0.034% and 0.059%, which corresponds to silver contents of 0.60, 3.95 and 6.0 ppm, respectively. For testing the leaching of nanoscaled silver from the polymer, the polymers were converted into plastic "coupons", and were referred to as 2.5%, 5% and 10% coupons. Each coupon was 3.44 x 2.31 inches with a total surface area of 15.8984 in^2.

The study design was based on FDA methodology designed for migration testing of food contact substances. Leaching of nanoscaled silver from the control, 2.5%, 5% and 10% plastic coupons was evaluated using the following food simulants: 1) acidic water (pH 2); basic water (pH 8); 3) 10% ethanol in water; 4)10% monosodium phosphate (salt) in water; 5) 10% oil in water; 6) 10% salt with 10% sugar in water; and 7) 10% salt with 10% sugar in water. For number 7 food stimulant migration was conducted on the coupon after physical abrasion. For testing, the coupons were placed in a Stomacher bag with 50 mL of the above food simulants and agitated at temperatures of 40 and 100°C for intervals of 24, 48, 96, 168 and 240 hours. For each type of coupon (control, 2.5%, 5% and 10%), a single coupon was tested for each food simulant at each temperature and duration of exposure.

Following exposure of the plastic coupons to the food stimulants, these food simulants were analyzed for silver concentrations using a standardized induction coupled plasma-atomic emission spectrophotometry (ICP-AES) method based on EPA Method 200.7. This method is designed for determining trace metals in water and solid waste samples, and it has a reported limit of quantitation (LOQ) of 0.002 mg/L for silver in aqueous samples. Although this is an accepted EPA method, no method validation data were provided to support the use of this method. Therefore, the adequacy of the analytical method under the conditions of this study could not be evaluated.

For the acidic and basic food simulants, concentrations of silver in the leachate from each type of plastic coupon (control, 2.5%, 5%, and 10%) were ≤0.002 mg/L at the end of each exposure period (24-240 hours) at both temperatures tested. This level equates to a migration value for silver of <6.29 ng/in^2. (using: *result x control x 0.00314 mg/in^2) = 0.002 x 0.00314 mg/in^2 = 6.28 x 10^{-6} mg/in^2 x 1 ng/1 x10^{-6} mg = 6.28 ng/in^2*

For the 10% oil food simulant, silver concentrations in all the leachate samples were also <0.002 mg/L, with only one exception. The leachate from the 2.5% coupon exposed for 240 hours at 100°C had silver residues of 0.021 mg/L, which correspond to a migration value of 65.9 ng/in^2.

For the 10% ethanol food simulant, silver concentrations in the leachate samples were <0.002-0.017 mg/L; however, 31 of the 40 samples having residues <0.002 mg/L.

For the samples with quantifiable silver residues, there were no apparent correlation between silver concentrations in the leachates and the levels of Nanosilva in the plastic, or with the temperature and duration of exposure. The maximum silver concentration found in the 10% ethanol leachate was from a 5% coupon exposed for 48 hours at 100 °C. Based on this concentration (0.017 mg/L), the maximum migration value for silver in the 10% ethanol food simulant was 53.4 ng/in^2.

For the 10% salt food simulant, initial analyses indicated that silver concentrations were <0.002-0.88 mg/L in all the leachate samples. However, the initial analyses also detected silver at levels of 0.002-0.004 mg/L in the control samples from both the 40°C and 100°C exposures. With the exception of the 48-hour samples, all the samples were retested. In the repeat analyses, residues at the 40°C exposure were <0.002-0.0088 mg/L, with 12 of the 20 samples having residues <0.002 mg/L. Repeat analyses of samples from the 100°C exposure, indicated that residues were <0.002-0.57 mg/L, with 13 of the 20 samples having residues <0.002 mg/L. The maximum silver residues found in the 10% salt leachate were from the 5% and 10% coupons exposed at 100°C for up to 240 hours. Silver residues in these leachates averaged 0.12 and 0.73 mg/L for the 5% and 10% coupons, respectively, after 240 hours of exposure. Although these data suggest that there is an increase in silver leaching with increasing Nanosilva concentrations in the plastic and with increasing durations of exposure, the residue data from the earlier sampling intervals (24-168 hours) showed no tend toward increasing residues with exposure time or with Nanosilva concentrations in the plastic. Based on the silver concentrations found in the 10% salt leachates after 240 hours at 100 °C, the maximum potential migration values for silver in the 10% salt food simulant would be 0.38 ug/in^2 for the 5% coupon and 2.38 µg/in^2 for the 10% coupon.

For the 10% salt/10% sugar food simulant, silver concentrations in the leachate samples were <0.002-0.014 mg/L, with only 8 of the 40 samples having quantifiable silver residues (0.0021-0.014 mg/L). There was no correlation between silver concentrations in the leachates and the levels of Nanosilva in the plastic, or with the temperature or duration of exposure. In fact, the two highest silver concentrations in any leachate were found in a control sample exposed at 40°C for 168 hours (0.014 mg/L), and a control sample exposed at 100°C for 96 hours (0.0058 mg/L). The maximum silver concentration from any treated coupon sample was 0.004 mg/L, which would equate to a migration value for silver of 12.6 ng/in^2.

The addition of an abrasion treatment to the 10% salt/10% sugar food simulant had no apparent affect on the leaching of silver from the plastic coupons. Silver concentrations in the leachates were <0.002-0.027 mg/L, with only 14 of the 40 samples having quantifiable silver residues. There was again no correlation between silver concentrations in the leachates and the levels of Nanosilva in the plastic, or with the temperature or duration of exposure. The maximum silver concentration for the 10% salt/10% sugar food simulant (with abrasion) was found in the leachate from a 5% coupon exposed at 40°C for 48 hours (0.027 mg/L). This concentration equates to a migration value for silver of 84.9 ng/in^2.

Considering all five types of food simulants tested, the leaching of silver from the plastic coupons was highest for the 10% salt food simulant, with maximum migration values of 0.38 and 2.38 μg of silver/in^2 from the 5% and 10% coupons, respectively, after 240 hours of exposure at 100°C. However, given the variability in the data and the lack of proper method validation, these values do not provide a scientifically explainable results. Regardless of the concentration of Nanosilva in the plastic coupons and the temperature and duration of expose to the food simulants, the migration values for silver were generally on the order of 7-50 ng/in^2 for leachate samples having quantifiable residues of silver.

A. BACKGROUND

The chemistry of silver ions has been known for quite sometime. The chemical and biochemical (biological) interactions of silver ions are known. The ionic form of silver (Ag^+) has long been utilized in medicine for its antimicrobial properties, particularly against bacteria and fungi. The antimicrobial mode of action of silver ions is believed to be based on its irreversible binding to membrane enzymes, resulting in the disruption of cell membrane functions.

Nanosilva, LLC has developed a new antimicrobial silver compound, and has been characterized by the registrants to: consists of particles of metallic silver (Ag^0), silver nanoparticles, that are covalently bound to sulfur, which in turn is covalently bound to a nano-silica particle through a propylsilyl bridge (see Tables A.1 and A.2). The overall size of the silver-sulfur-silica particle is 30-50 nm, with the individual silver clusters being 2-3 nm in size. For comparison, the diameter of a silver ion as bulk material is 0.288 nm. While the antimicrobial action of silver ions is direct, the mode of action for the nano-silver-silica complex as claimed by Nanosilva is indirect. The reported antimicrobial mode of action for the nano-silver particles involves the silver acting as a catalyst for the dissociation of molecular oxygen in the environment. The resulting reactive oxygen radicals *(ROS mechanism)* either stabilize back into molecular oxygen or damage cell walls/membranes of an organism with which it comes into contact. Therefore, the antimicrobial effect of the nano-silver particles occurs only in the presence of oxygen.

NanoSilva, LLC has proposed incorporating nano-silver-silica particles into a wide-variety of materials in order to provide surface antimicrobial activity. Some of the proposed uses for NanoSilva™ include coatings for medical equipment, textiles, house wares, paints, building materials, food and beverage packaging, storage containers and processing equipment.

The end-use product developed by the petitioner for use in the manufacture of materials containing nano-silver-silica particles is an aqueous colloidal suspension. The formulation contains approximately 40.4% of the nano-silver-silica particles by weight, with an actual silver content of approximately 1.19%. For purposes of this report, the active ingredient is considered to consist of the metallic silver.

The registrants have proposed to use their product which consists of nano-silver-silica particles into food and beverage packaging, and on food contact surfaces, which has the potential for migration of nano-silver into foods. To evaluate the potential for the migration of nano-silver from food contact surfaces (FCS) into foods, the petitioner has submitted a study examining the leaching of nano-silver into various simulated foods from a plastic formulated with the nano-silver-silica particles.

Table A.1 summarizes the basic physical/chemical characteristics of the product

CBI

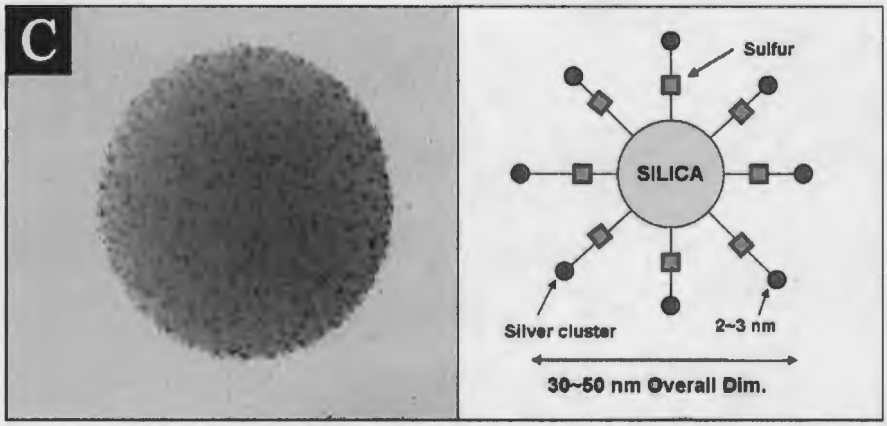
TABLE A.1. Nanosilva Nomenclature.	
Compound	 TEM image of Nanosilva™ Complex Particle Diagram of silver-silica particle
Common name	Silver-sulfur-silica complex
Company experimental name	NSPW-L30SS
IUPAC name	Silver
CAS name	Silver
CAS registry number	7440-22-4
End-use product (EP)	NanoSilva™; silver-silica particles in an aqueous colloid, containing 1.19% silver by wt.

TABLE A.2. Physicochemical Properties of the Nano-Silver-Silica Colloid *		
Parameter	Value	Reference (MRID#)
Melting point (°C)	Product is slurry	[redacted]
pH	7.8 (average of 5 lots)	478289-13
Density	1.1179 kg/l	478289-16
Water solubility	Insoluble to water (< 100 ppb)	478289-17
Solvent solubility	Not provided	[redacted]
Vapor pressure	Product is an aqueous slurry	[redacted]
Dissociation constant, pKa	Not applicable	[redacted]
Octanol/water partition coefficient, Log(Kow)	Product is an inorganic aqueous slurry	[redacted]
UV/visible absorption spectrum	Not provided	[redacted]

**** From Memo by Earl Goad ( )***

B. MATERIALS AND METHODS

B.1. Study Design

The design of the leaching study was based upon FDA methodology which is used for migration testing and analytical methods for food contact substances (From the submitted study: Sections II.D.1 through II.D.3, and Appendix II, Part 4 – Articles intended for repeated use). A linear low-density polyethylene (LLDPE) polymer was selected as the test material for evaluating the leaching of the nano-silver-silica particles as levels of migrants from LLDPE are generally higher than from high-density polyethylene (HDPE) or polypropylene (PP). The LLDPE polymer was tested using an

untreated control and three different concentrations of the Nanosilva colloid solution in the plastic. The target concentrations for the colloid formulation in the polymer were reported to be 0.00125%, 0.0025% and 0.005% (by weight). Based on a silver concentration of ~1.19% for the colloid, these levels of the colloid in the polymer are equivalent to silver concentrations of approximately 0.000015%, 0.00003% and 0.00006%, respectively, or 0.15, 0.3 and 0.6 ppm of silver in the polymer test material.

Leaching of silver from the polymer test materials was tested over time (24, 48, 96, 168 and 240 hours) at two temperatures (40 and 100°C) using plastic "coupons" that were exposed to the following food simulants: 1) acidic water (pH 2); 2) basic water (pH 8); 3) 10% ethanol in water; 4) 10% monosodium phosphate in water; 5) 10% oil in water; 6) 10% salt with 10% sugar in water; 7) and 10% salt with 10% sugar in water (for this part of the study, physical abrasion was done on the coupons). Following exposure, samples of the food simulants were analyzed for silver content using ICP-AES. These techniques provided total silver content without taking into account the nature of original silver content.

The description of the end-use product used for incorporation into the LLDPE polymer is provided in Appendix I. Based on the statement of formula, the final colloidal suspension contains ~40.4% of the nano-silver-silica particles and ~1.19% of silver. Assuming that all water would be lost from the formulation during incorporation and formation into the plastic polymer, the remaining nano-silver-silica material would have a silver content of ~2.42%. A description of the colloid formulation and its incorporation into the plastic coupons which were used in the leaching study were provided in Addendum 7 of the study report.

The initial step in the production of the plastic coupons involved preparation of a "master batch" of LLDPE that reportedly contained 5% of the nano-silver-silica colloid suspension. For example, to prepare the 2 lb master batch, the petitioner calculated that 19.636 g of the colloidal formulation should be blended with 907.2 g of granular LLDPE (Novapol® Polyethylene Resin). The amount of the colloid used was then reportedly doubled (39.27 g) to presumably account for water loss during formation of the plastic pellets. However, the subsequent instructions indicate that the polymer resin was blended with only 19.636 g of colloidal formulation (Study Report page 916). Therefore, it is unclear whether 19.636 or 39.27 g of the colloid formulation was actually used, although the addition of 39.27 g of colloid would most closely approximate the reported 5% concentration in the master batch. However, this contradictory approach has created a big uncertainty in the analyses and interpretations of the study results. After blending the colloid and resin, the master batch mix was compounded using a single-screw extruder with a barrel temperature profile of 180°C. The extruded material was then pelletized and dried for further compounding and injection molding.
Although the master batch was reported to contain 5% of the colloid, the actual amount of the colloid in the plastic was apparently either 2.1% or 4.2%, depending on which amount was actually added to the resin.

The pelletized master batch resin was then blended with additional LLDPE at master batch ratios of 2.5%, 5% or 10%, and the blended samples were injection molded into standard chips using an injection-molding machine with a barrel temperature of 180°C. Each coupon measured 3.44" x 2.31" with a thickness of 0.83", and weighed approximately 11.2 grams. The resulting plastic coupons are referred to as 2.5%, 5% or 10% coupons throughout the report, although these percentages do not reflect the actual amount of the colloid in the coupons.

We calculated the actual *percent (%)* of the colloid formulation and the *percent (%*)silver in the final plastic coupons and these are reported in Table B.1. Assuming that the master batch contains 5% of the colloid, the final 2.5%, 5% and 10% batches of coupons would contain 0.125%, 0.25% and 0.50% colloid, respectively, or 0.0015%, 0.003% and 0.006% silver (15, 30 and 60 ppm).

However, at the beginning of the study report (page 17), the study author also indicated that the composition of the final plastic coupons at the three levels tested was 0.00125%, 0.0025% or 0.005% active concentration (colloid). These concentrations would equate to final silver concentrations of 0.000015%, 0.00003%, and 0.00006%, respectively, or 0.15, 0.3 and 0.6 ppm of sliver. These concentrations are 100x lower than the levels based on the manufacturing information in Addendum 7.

Table B.1. Description of Polymer Coupons used for Leaching Study

Coupon ID [1]	% colloid in master batch	% master batch in final plastic [1]	% colloid in final plastic coupon [2]	% silver in colloid [3]	Silver content of plastic coupons [2]	
					% wt. [2]	ppm [4]
5% Colloid in Master Batch [1]						
LLDPE 2.5%	5 .0	2.5	0.125	1.19	0.0015	15
LLDPE 5.0%	5.0	5.0	0.250	1.19	0.0030	30
LLDPE 10%	5.0	10	0.500	1.19	0.0060	60
2.1% Colloid in Master Batch [2]						
LLDPE 2.5%	2.1	2.5	0.053	1.19	0.00063	6.3
LLDPE 5.0%	2.1	5.0	0.105	1.19	0.00125	12.5
LLDPE 10%	2.1	10	0.210	1.19	0.0025	25
4.2% Colloid in Master Batch [2]						
LLDPE 2.5%	4.2	2.5	0.105	1.19	0.00125	12.5
LLDPE 5.0%	4.2	5.0	0.210	1.19	0.0025	25
LLDPE 10%	4.2	10	0.420	1.19	0.0050	50

[1] As reported by the registrants.
[2] As calculated by the reviewer.
[3] Calculated from statement of formula., submitted by the registrants
[4] Calculated by reviewer (%silver x 10,000).

To obtain the ppm of silver in the coupon, following equation was used by the reviewer:

% colloid in master batch x % master batch in final plastic x % silver in colloid = ppm of silver in the coupon

(5 x .025= 0.125; 0.125 x 0.0119= 0.001475 ~ 0.0015)

To verify the silver content of the manufactured polymer coupons, four plastic coupons from each treatment level were analyzed by Florida – Spectrum Environmental

Services (Fort Lauderdale, FL). Sections were cut from each of the four corners of each coupon to obtain a ~5 g sample for each coupon. Each sample was then dry ashed (according to AOAC Method 900.02 (44.105), 17th Edition), and the resulting ash was acidified with 20 mL of concentrated nitric acid/ water (50:50), and brought to final volume of 100 mL with nitric acid/ water (10:90). The samples were then analyzed by ICP-AES using EPA Method 200.7. The reported method LOQ was 0.0006 mg/L, which equates to an LOQ of 0.012 ppm based on the 5 g sample analyzed.

The results from the analyses are reported in Table B.2. We noted that no method validation data were provided to validate the adequacy of the method for the analysis of silver in treated polymer coupons.

Table B.2. Silver Content of Manufactured NanoSilva Polymer Coupons as Determined by ICP-AES.

Polymer Treatment	Sample ID	Measured silver concentration (mg/L)	Silver content of coupons (mg/kg)	Average silver content (mg/kg)	Std. Dev.
Control Coupons	G1326	<0.0006 [1]	<0.012 [1]	<0.012	0
	G1326	<0.0006	<0.012		
	G1326	<0.0006	<0.012		
	G1326	<0.0006	<0.012		
2.5% Coupons	G1327	0.04	0.8	0.60	0.20
	G1327	<0.0006 [2]	<0.012 [2]		
	G1327	0.02	0.4		
	G1327	0.03	0.6		
5% Coupons	G1328	0.20	4.0	3.95	0.50
	G1328	0.17	3.4		
	G1328	0.19	3.8		
	G1328	0.23	4.6		
10% Coupons	G1329	0.35	7.0	7.00	1.28
	G1329	0.30	6.0		
	G1329	0.31	6.2		
	G1329	0.44	8.8		

[1] The method LOQ for silver was reported to be 0.0006 mg/L, which is equivalent to a LOQ of 0.012 ppm for the 5 g coupon sample.

[2] This value was excluded from the average as it was judged to be an outlier.

No silver was detected in the control coupons, and the average silver content in the 2.5%, 5% and 10% coupons was 0.6, 3.95 and 7.0 ppm, respectively. The silver content of the two highest levels were proportional to their treatment levels, but the silver content of the lowest level tested was 6.6x lower than the next highest level.

The silver content of the 2.5%, 5% and 10% coupons were 0.00006%, 0.0004% and 0.0007%, respectively. Based these concentrations and the silver content (1.19%) of the nano-silver-silica colloid, the 2.5%, 5% and 10% coupons would have actual colloid concentrations of 0.005%, 0.034%, and 0.059%, respectively.

Based on the experimentally determined silver content of the nanosilva treated polymer coupons, we have noted that the colloid content (%) of the test coupons is approximately 10x higher than the amount reported at the beginning of the study (page 17), but is also

10x lower then the colloid concentrations determined from the manufacturing information in Addendum 7 of the report.

B.3. Food Simulants

To assess leaching of the nano-silver-silica particles from the treated LLDPE coupons, the following food simulants were utilized: acidic water, adjusted to pH 2.0 ± 0.1 with 10% nitric acid; basic water, adjusted to pH 8.0 ± 0.1 with 0.1N NaOH; water containing 10% ethanol; an aqueous 10% salt solution prepared using monobasic sodium phosphate; an aqueous solution containing 10% salt and 10% sugar prepared using monobasic sodium phosphate and sucrose; and an aqueous emulsion containing 10% extra virgin olive oil. These simulants were selected in accordance with the recommendations by the FDA for determination of migration factors for food contact substances. However, the FDA guidance also recommends the inclusion of an aqueous 50% ethanol solution for testing of migration.

B.4. Test System

Control and treated polymer coupons (3.44 x 2.31 x 0.083 inches) were subjected to leaching using the above five food simulants at two temperatures for up to 10 days (240 hours). A total for 44 plastic coupons (11 coupons per Nanosilva treatment level) were utilized for the entire study. For each combination of food simulant, temperature, and exposure duration, one coupon from each treatment (control, 2.5%, 5% and 10%) was subjected to any given leaching treatment. The leaching treatments were <u>not</u> replicated as recommended by FDA guidance. In addition, the same plastic coupons were reused repeatedly for different leaching treatments over the course of the study.

The plastic coupons from each Nanosilva treatment level were subjected to leaching at 40 °C and 100 °C using each of the five food simulants for periods of 24, 48, 96, 168 and 240 hours. Single control, 2.5%, 5% and 10% coupons were used for each food simulant at each temperature and time point. For treatment, the plastic coupon was placed into an individually labeled Stomacher bag with 50 ml of the appropriate food simulant, such that both sides of the coupon were exposed to the food simulant. The air was removed and the bag was heat sealed. For the 40°C treatment, the bags were placed on a mechanical shaker in an incubator that maintained the temperature at 40 ± 2°C for the duration of exposure. For the 100°C treatment, the bags were placed in a shaking water bath, which maintained the temperature at 100 ± 2°C.

An additional treatment was also conducted in which the four types of coupons (control, 2.5%, 5% and 10%) were exposed to 50 mL of the 10% salt/10% sugar food simulant at 40 and 100 °C at intervals up to 240 hours in conjunction with a scrubbing treatment. For this treatment, two 2.5 x 3.5 inch sections of plastic scrubbing pads were placed on either side of the plastic coupon in the Stomacher bag. The plastic coupon was then scrubbed by applying normal, firm pressure for 1 minute and the scrubbing pads were left in the bag with the coupon for the duration of the exposure (24, 48, 96, 168 or 240 hours). (Note: This is the physical abrasion technique used in this study) The

methods directions were unclear, but it appeared that the coupons were also scrubbed at the end of the expose time prior to sampling the leachate.

For each type of food simulant, six additional control plastic coupons were also placed into individual Stomacher bags, diluted with 50 mL of the appropriate food simulant, and then spiked with standard silver nitrate solutions at three fortifications levels. The bags were sealed and the fortified controls were exposed for 240 hours at 40°C or 100°C (3 fortified samples per temperature). The fortification levels for silver tested were 20, 100 and 200 ppm for the acidic and basic water simulants, and 0.5, 1.0 and 5.0 ppm for the remaining food simulants.

B.5. Sample Collection, Handling and Preparation

For each food simulant treatment, the entire 50 mL leachate was collected from each sample at the end of each exposure period (24, 48, 96, 168 and 240 hours). For the pH 2 and pH 8 water samples and the 10% salt samples, the leachates were acidified with concentrated nitric acid and stored in HDPE containers until ICP-AES analysis. For the 10% ethanol samples, the leachates were first evaporated in a drying oven, redissolved in 10% nitric acid, heated for 1 hour and then brought back to a 50 mL volume with water before storage. For the remaining food simulants (10% sugar and salt, and 10% oil), each sample was evaporated to dryness in crucibles in a drying oven and then ashed in a furnace. The resulting ash was dissolved in 50 mL of 10% nitric acid, heated for 1 hour, and then brought back to a 50 mL volume with water. The exposure tests, sample collection, and sample preparations were conducted by KAPPA Laboratories, but the prepared samples were sent out to other laboratories for silver analysis.

A summary of the sample storage conditions and durations was not provided in the study report; however, information from the analytical labs suggests that the prepared samples were stored under refrigeration (1-5°C) until analysis.

Analysis of the acidic and basic water samples, 10% ethanol samples, 10% salt solution samples, and 10% salt/10% sugar samples was conducted by KSA Environmental Laboratory (Miramar, FL), and analysis of the 10% oil samples was conducted by Xenco Laboratories (Miami Lakes, FL).

B.6. Analytical Method

The prepared leachate samples were analyzed for silver concentration using a standardized ICP-AES method based on EPA Methods 200.7 and 6010B, for determining trace metals in water and solid waste samples. The analytical laboratories reported various LODs and LOQs for silver in the prepared aqueous samples, ranging from 0.0008-0.240 mg/L for the LOD and 0.010-0.500 mg/L for the LOQ. Because no data were provided validating the method at the listed LOQs, the preliminary LOQ (0.002 mg/L) reported for Method 200.7 was used as the method LOQ for reporting all analyses in this report.

The study report did not provide standard curves for instrument calibration from any of the laboratories, and concurrent procedural recoveries for silver from the various samples were not performed. Therefore, the adequacy of the analytical method under the conditions of this study could not be evaluated.

B.7. Data Analysis

Migration values for silver from the plastic coupons were calculated for each type of coupon and exposure treatment (using: food simulant x temperature x exposure duration). The migration values were calculated using the following formula:

Migration value (mg/in^2) = (silver residues mg/L)*(0.05 L of leachate/15.8984 in^2)

The study author also reported "average" migration values for each combination of coupon type x food simulant x temperature. However, these values were not included in this report as the averages were inappropriately determined using the different exposure intervals. None of the test treatments were replicated; therefore, averaging of the data is inappropriate.

C. RESULTS AND DICUSSION

C.1. Experimental Design

The study was generally conducted in accordance with the existing FDA guidance for determining migration of substances from food contact substances. The plastic polymer utilized for incorporation of the Nanosilva test substance was an LLDPE plastic as recommended. The plastic coupons were evaluated at the recommended temperatures (40 and 100°C) and durations of exposure (5 intervals from 24 to 240 hours), and the food simulates tested are representative of a wide variety of food types. However, the study design did deviate from FDA guidance in several areas.
For example: 1) a food simulant volume of 50 mL was used in the current study; whereas, FDA guidance recommends using a minimum volume of 10 mL/in^2 of the FCS. For the current study, this would equate to a volume of ~160 mL for the food simulant. (2) The inadequate characterization of the test materials; (3) the lack of replication in the exposure tests; and (4) the lack of adequate method validation data.

In addition to the factors noted above for the flaws in the study design, A) it is unclear to us exactly what is the content (%) of the nano-silver-silica particles in the manufactured plastic coupons used for testing. The percentages given at the beginning of the report do correspond to the percentages calculated by the Agency based on the available manufacturing information, and neither of these percentages corresponds to the experimentally determined value based on the measured silver content of the various plastic coupons.

B) For the four types of plastic coupons tested (control, 2.5%, 5% and 10%), each exposure test, consisting of a given food simulant x temperature x exposure duration, was conducted only once. FDA methodology recommends that each test should be conducted in triplicate. The study authors assumed that the three different types of coupons represented experimental replicates; however, Agency believes that coupons containing different levels of the nano-silver-silica particles clearly can not be treated as replicates.

C) The capability of the analytical procedures to recover silver from the food simulants was not adequately validated. The only recovery tests conducted involved fortifying the food simulants with silver prior to exposure of the control coupons at 100°C for 240 hours. Although the recoveries from these fortification tests are of scientific interest, they do not demonstrate whether or not silver residues are adequately recovered from the food simulants at the end of the exposure period. To demonstrate the adequacy of the procedures used for sample preparation and analysis, the control food simulate samples should be fortified at the end of the exposure period, as specified in the FDA methodology.

D) In addition, the fortification levels used for method validation should approximate the potential levels to be measured in the food simulants (0.5x, 1x and 2x fortification levels). In the current study, the fortification levels used for silver (0.5-200 mg/L) were well in excess of the measured silver concentrations, which were generally on the order of <0.002-0.02 mg/L.

C.2. Analytical method

Although the sample preparation procedures and ICP-AES analytical method used for the analysis of silver in the food simulants is based on accepted EPA methods for determining trace metals in aqueous and solid waste samples (Methods 200.7 and 6010B), the analytical procedures were not validated in conjunction with the current study as required by FDA guidance. As indicated above, the adequate recovery of silver from the food simulants should be demonstrated by fortification of control food simulants at the end of the exposure period using fortification levels similar to the expected levels of residues. Based on the expected levels of silver in the food simulants, samples should have been fortified with silver at levels of 0.002-0.10 mg/L at the end of the exposure period. Given that numerous samples had silver residues <LOQ, it is particularly important to validate the method at the reported LOQ. Acceptable average recoveries for the method procedures should range from 60-110% with relative standard deviations of <20%.

Although the recoveries from the control sample fortifications used in the current study are not acceptable for assessing the adequacy of method recovery, the low recoveries (<70%) of silver from several matrices and the wide variability in the recovery values suggest that the quantitative recovery of silver from the food simulants may be problematic, particularly at lower levels as Table C.2.1 indicates. However, given that the samples were fortified with silver prior to long term exposure (240 hours) at 100°C, the low recoveries may reflect the binding of the silver (and the nature of silver) to the either the plastic coupon or Stomacher bag during exposure.

In addition, to providing acceptable method validation data, standard calibration curves should have been provided by the analytical laboratories as specified by the FDA methodology. In fact for all such studies, absence of standard calibration creates doubts about the scientific soundness of a study

TABLE C.2.1. Summary of Recoveries of Silver from Fortified Leachates with Control Coupon Samples following Exposure to Various Food Simulants at 100°C for 240 hours.

Food stimulant	Exposure temp.	Spike Level (ppm)	Sample ID Number	Silver concentration (ppm)	Recoveries (%)
KSA Environmental Laboratory					
pH 2.0 water	40°C	20	I934	22	110
		100	I935	93	93
		200	I936	200	100
	100 °C	20	I931	11	55
		100	I932	92	92
		200	I933	190	95
pH 8.0 water	40 °C	20	I940	20	100
		100	I941	100	100
		200	I942	200	100
	100 °C	20	I937	19	95
		100	I938	100	100
		200	I939	200	100
10% EtOH	40 °C	0.5	I1056A	0.6	120
		1.0	I1056 B	0.69	69
		5.0	I1056C	1.7	34
	100 °C	0.5	I1057 A	0.18	36
		1.0	I1057B	0.33	33
		5.0	I1057C	0.059	1.2
10% NaH_2PO_4	40 °C	0.5	I1058A	0.87	174
		1.0	I1058B	0.89	89
		5.0	I1058C	4.0	80
	100°C	0.5	I1059A	0.41	82
		0.5	I1260	0.31	62
		1.0	I1059B	0.63	63
		5.0	I1059C	3.1	62
10% sugar/ 10% salt	40 °C	0.5	I1194A	0.46	92
		1.0	I1194B	0.34	34
		5.0	I1194C	0.68	13.6
	100°C	0.5	I1196A	0.068	13.6
		1.0	I1196B	0.94	94
		5.0	I1196C	0.12	2.4
10% sugar/ 10% salt, with scrubbing	40 °C	0.5	I1195A	0.02	4
		1.0	I1195B	0.068	6.8
		5.0	I1195C	0.18	3.6
	100°C	0.5	I1197A	0.021	4.2
		1.0	I1197B	0.015	1.5
		5.0	I1197C	1.8	36

TABLE C.2.1. Summary of Recoveries of Silver from Fortified Leachates with Control Coupon Samples following Exposure to Various Food Simulants at 100°C for 240 hours.					
Food stimulant	Exposure temp.	Spike Level (ppm)	Sample ID Number	Silver concentration (ppm)	Recoveries (%)
Xenco Laboratories					
10% olive oil	40 °C	0.5	I1261A	0.039	7.8
		1.0	I1261B	0.057	5.7
		5.0	I1261C	0.370	7.4
	100 °C	0.5	I1262A	0.014	2.8
		1.0	I1262B	0.11	11
		5.0	I1262C	0.30	6.0

C.2a: Calculations:

A number of calculation steps are involved in the study and a summary of these calculations are provided here:

1. ***Migration value (mg/in^2) = (silver residues mg/L)*(0.05 L of leachate/15.8984 in^2)***
2. ***(result x control x 0.00314 mg/in^2) = 0.002 x 0.00314 mg/in^2 = 6.28 x 10^{-6} mg/in^2 x 1 ng/1 x10^{-6} mg = 6.28 x ng/in^2***
3. ***For Table B.1(5 x .025= 0.125; 0.125 x 0.0119= 0.001475 ~ 0.0015); % colloid in master batch x % master batch in final plastic x % silver in colloid = ppm of silver in the coupon***

4. *If a one liter of solution (assuming density = 1.08 g/ml) is mixed with 110 lbs of polymer resin, then this is taking 9.818 g of solution /lb of resin; since a sample of 2 lbs of master batch of resin was used, this amounts to 9.818 g x 2 = 19.636 g of solution mixture; that is:*
1000ml x 1.08 g/ml; 1080 g solution/110lbs = 9.818 g/lb x 2lb = 19.636 g

C.3. Migration Testing Results

For the acidic and basic food simulants, concentrations of silver in the leachate from all four types of plastic coupons (control, 2.5%, 5%, and 10%) were ≤0.002 mg/L at the end of each exposure period (24-240 hours) for both temperatures tested (Tables C.3.1 and C.3.2). This level equates to a migration value for silver of <6.29 x 10^{-6} mg/in^2, or <6.29 ng/in^2.

For the 10% ethanol food simulant, silver concentrations in all the leachate samples were <0.002-0.017 mg/L, with 31 of the 40 samples having residues <0.002 mg/L (Table C.3.3). In addition, retesting of five samples, with quantifiable residues of 0.0027-0.011 mg/L, returned results of <0.002 ppm. For the samples with quantifiable residues, there were no apparent correlation between silver concentrations in the leachates and the levels of Nanosilva in the plastic, or with the temperature or duration of exposure. The maximum silver concentration found in the 10% ethanol leachate was from a 5% coupon exposed for 48 hours at 100 °C. Based on this concentration (0.017

mg/L), the maximum migration value for silver in the 10% ethanol food simulant was 53.4 x 10^{-6} mg/in^2, or 53.4 ng/in^2.

For the 10% salt food simulant, initial analyses indicated that silver concentrations were <0.002-0.88 mg/L in all the leachate samples (Table C.3.4). Only 6 samples had residues <0.002 mg/L, and they were all from the tests using the 40°C exposure. However, the initial analyses also detected silver at levels of 0.002-0.004 mg/L in the control samples from both the 40°C and 100°C exposures. With the exception of the 48-hour samples, all the samples were retested. In the repeat analyses, residues at the 40°C exposure were <0.002-0.0088 mg/L, with 12 of the 20 samples having residues <0.002 mg/L. Repeat analyses of samples from the 100°C exposure, indicated that residues were <0.002-0.57 mg/L, with 13 of the 20 samples having residues <0.002 mg/L. For the tests using the 40°C exposure, there were no apparent correlation between silver concentrations in the leachates and the levels of Nanosilva in the plastic or in the duration of exposure. For the 100°C tests, there were also no apparent correlation between residues in the leachates and the levels of Nanosilva in the plastic or the duration of exposure, with two exceptions. For both the 5% and 10% coupons exposed at 100°C, the maximum residues in the leachate were detected at the longest exposure interval (240 hours). The silver residues averaged 0.12 and 0.73 mg/L for the 5% and 10% coupons, respectively, after 240 hours. These data suggest that there is an increase in silver leaching with increasing Nanosilva concentrations in the plastic and with increasing durations of exposure. However, the residue data from the earlier sampling intervals (24-168 hours) showed no tend toward increasing residues with exposure time or with Nanosilva concentrations in the plastic. Based on the maximum silver concentrations found in the 10% salt leachates after 240 hours at 100 °C, the maximum potential migration values for silver in the 10% salt food simulant would be 377 x 10^{-6} for the 5% coupon and 2280 x 10^{-6} mg/in^2 for the 10% coupon, or 0.38 and 2.38 μg/in^2.

For the 10% oil food simulant, silver concentrations in all the leachate samples were <0.002 mg/L, with only one exception (Table C.3.5). The leachate from a 2.5% coupon exposed for 240 hours at 100°C had silver residues of 0.021 mg/L. This concentration equates to a migration value of 65.9 x 10^{-6} mg/in^2, or 65.9 ng/in^2.

For the 10% salt/10% sugar food simulant, silver concentrations in the leachate samples were <0.002-0.014 mg/L (Table C.3.6). Only 8 of the leachate samples had quantifiable silver residues (0.0021-0.014 mg/L), and there was no correlation between silver concentrations in the leachates and the levels of Nanosilva in the plastic, or with the temperature or duration of exposure. In fact, the two highest silver concentrations in any leachate were found in a control sample exposed at 40°C for 168 hours (0.014 mg/L), and a control sample exposed at 100°C for 96 hours (0.0058 mg/L). The maximum silver concentration from any treated coupon sample was 0.004 mg/L, which would equate to a migration value of 12.6 x 10^{-6} mg/in^2, or 12.6 ng/in^2.

The addition of an abrasion treatment to the 10% salt/10% sugar food simulant had no apparent affect on the leaching of silver from the plastic coupons. For these

treatments, silver concentrations in the leachates were <0.002-0.027 mg/L, with only 14 of the 40 samples having quantifiable silver residues (Table C.3.7). There was again no correlation between silver concentrations in the leachates and the levels of Nanosilva in the plastic, or with the temperature or duration of exposure. The maximum silver concentration for the 10% salt/10% sugar food simulant (with abrasion) was found in the leachate from a 5% coupon exposed at 40°C for 48 hours (0.027 mg/L). This concentration equates to a migration value for silver of 84.9×10^{-6} mg/in^2, or 84.9 ng/in^2.

Considering all five types of food simulants tested, the leaching of silver from the plastic coupons was highest for the 10% salt food simulant, with maximum migration values of 0.38 and 2.38 µg of silver/in^2 from the 5% and 10% coupons, respectively, after 240 hours of exposure at 100°C. However, given the variability in the data and the lack of proper method validation, these values are questionable. Regardless of the concentration of Nanosilva in the plastic coupons and the temperature and duration of expose to the food simulants, the migration values for silver were generally on the order of 7-50 ng/in^2 for leachate samples having quantifiable (>0.002 mg/L) residues of silver.

2 temps x 5 times x 4 coupons = 40

TABLE C.3.1. Residue Data from Migration Testing of NanoSilva Particles in LLDPE Exposed to the Food Simulant of Acidic Water (pH 2.0).

Test Sample	Exposure Temperature (°C)	Exposure Duration (hours)	Sample ID	Silver concentration in Leachate (ppm) [1]	Migration Value [2] (mg/in^2)
Control Coupon	40	24	1860A	<0.002	$<6.29 \times 10^{-6}$
		48	1876A	<0.002	$<6.29 \times 10^{-6}$
		96	1905A	<0.002	$<6.29 \times 10^{-6}$
		168	1910A	<0.002	$<6.29 \times 10^{-6}$
		240	1927A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	40	24	1860B	<0.002	$<6.29 \times 10^{-6}$
		48	1876B	<0.002	$<6.29 \times 10^{-6}$
		96	1905B	<0.002	$<6.29 \times 10^{-6}$
		168	1910B	<0.002	$<6.29 \times 10^{-6}$
		240	1927B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	40	24	1860C	<0.002	$<6.29 \times 10^{-6}$
		48	1876C	<0.002	$<6.29 \times 10^{-6}$
		96	1905C	<0.002	$<6.29 \times 10^{-6}$
		168	1910C	<0.002	$<6.29 \times 10^{-6}$
		240	1927C	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	40	24	1860D	<0.002	$<6.29 \times 10^{-6}$
		48	1876D	<0.002	$<6.29 \times 10^{-6}$
		96	1905D	<0.002	$<6.29 \times 10^{-6}$
		168	1910D	<0.002	$<6.29 \times 10^{-6}$
		240	1927D	<0.002	$<6.29 \times 10^{-6}$
Control Coupon	100	24	1858A	<0.002	$<6.29 \times 10^{-6}$
		48	1874A	<0.002	$<6.29 \times 10^{-6}$
		96	1903A	<0.002	$<6.29 \times 10^{-6}$
		168	1908A	<0.002	$<6.29 \times 10^{-6}$
		240	1926A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	100	24	1858B	<0.002	$<6.29 \times 10^{-6}$
		48	1874B	<0.002	$<6.29 \times 10^{-6}$
		96	1903B	<0.002	$<6.29 \times 10^{-6}$
		168	1908B	<0.002	$<6.29 \times 10^{-6}$
		240	1926B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	100	24	1858C	<0.002	$<6.29 \times 10^{-6}$
		48	1874C	<0.002	$<6.29 \times 10^{-6}$
		96	1903C	<0.002	$<6.29 \times 10^{-6}$
		168	1908C	<0.002	$<6.29 \times 10^{-6}$
		240	1926C	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	100	24	1858D	<0.002	$<6.29 \times 10^{-6}$
		48	1874D	<0.002	$<6.29 \times 10^{-6}$
		96	1903D	<0.002	$<6.29 \times 10^{-6}$
		168	1908D	(0.0012) [3]	$<6.29 \times 10^{-6}$
		240	1926D	(0.0011) [3]	$<6.29 \times 10^{-6}$

[1] The reported LOQ of Method 200.7 is 0.002 ppm for aqueous silver; any reported values <LOQ are listed in parentheses.

[2] For samples with silver residues <LOQ, the LOQ (0.002 ppm) was used for calculating a maximum potential migration value.

[3] The registrants reported that as the result was less than the LOQ of the method these samples were retested, but the we could find no data

TABLE C.3.2. Residue Data from Migration Testing of NanoSilva Particles in LLDPE Exposed to the Food Simulant of Basic water (pH 8.0).

Test Sample	Exposure Temperature (°C)	Exposure Duration (hours)	Sample ID	Silver concentration in Leachate (ppm) [1]	Migration Value [2] (mg/in^2)
Control Coupon	40	24	1859A	<0.002	$<6.29 \times 10^{-6}$
		48	1875A	<0.002	$<6.29 \times 10^{-6}$
		96	1904A	<0.002	$<6.29 \times 10^{-6}$
		168	1909A	<0.002	$<6.29 \times 10^{-6}$
		240	1925A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	40	24	1859B	<0.002	$<6.29 \times 10^{-6}$
		48	1875B	0.002	6.29×10^{-6}
		96	1904B	<0.002	$<6.29 \times 10^{-6}$
		168	1909B	<0.002	$<6.29 \times 10^{-6}$
		240	1925B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	40	24	1859C	<0.002	$<6.29 \times 10^{-6}$
		48	1875C	<0.002	$<6.29 \times 10^{-6}$
		96	1904C	<0.002	$<6.29 \times 10^{-6}$
		168	1909C	<0.002	$<6.29 \times 10^{-6}$
		240	1925C	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	40	24	1859D	<0.002	$<6.29 \times 10^{-6}$
		48	1875D	<0.002	$<6.29 \times 10^{-6}$
		96	1904D	<0.002	$<6.29 \times 10^{-6}$
		168	1909D	<0.002	$<6.29 \times 10^{-6}$
		240	1925D	<0.002	$<6.29 \times 10^{-6}$
Control Coupon	100	24	1857A	<0.002	$<6.29 \times 10^{-6}$
		48	1873A	<0.002	$<6.29 \times 10^{-6}$
		96	1902A	<0.002	$<6.29 \times 10^{-6}$
		168	1907A	<0.002	$<6.29 \times 10^{-6}$
		240	1924A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	100	24	1857B	<0.002	$<6.29 \times 10^{-6}$
		48	1873B	<0.002	$<6.29 \times 10^{-6}$
		96	1902B	<0.002	$<6.29 \times 10^{-6}$
		168	1907B	<0.002	$<6.29 \times 10^{-6}$
		240	1924B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	100	24	1857C	<0.002	$<6.29 \times 10^{-6}$
		48	1873C	<0.002	$<6.29 \times 10^{-6}$
		96	1902C	<0.002	$<6.29 \times 10^{-6}$
		168	1907C	<0.002	$<6.29 \times 10^{-6}$
		240	1924C	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	100	24	1857D	<0.002	$<6.29 \times 10^{-6}$
		48	1873D	<0.002	$<6.29 \times 10^{-6}$
		96	1902D	<0.002	$<6.29 \times 10^{-6}$
		168	1907D	<0.002	$<6.29 \times 10^{-6}$
		240	1924D	<0.002	$<6.29 \times 10^{-6}$

[1] The reported LOQ of Method 200.7 is 0.002 ppm for aqueous silver; any reported values <LOD are listed in parentheses.

[2] For samples with silver residues <LOQ, the LOQ (0.002 ppm) was used for calculating a maximum potential migration value.

TABLE C.3.3. Residue Data from Migration Testing of NanoSilva Particles in LLDPE Exposed to the Food Simulant of 10 % EtOH.

Test Sample	Exposure Temperature (°C)	Exposure Duration (hours)	Sample ID	Silver concentration in Leachate (ppm) [1]	Migration Value [2] (mg/in^2)
Control Coupon	40	24	1970A	<0.002, (0.0013), <0.002	$<6.29 \times 10^{-6}$
		48	1976A	(0.0013)	$<6.29 \times 10^{-6}$
		96	1989A	(0.0009), <0.002	$<6.29 \times 10^{-6}$
		168	I1016A	(0.0014), <0.002	$<6.29 \times 10^{-6}$
		240	I1052A	0.0034, <0.002	8.49×10^{-6}
2.50% Coupon	40	24	1970B	<0.002, 0.004, 0.0027	9.12×10^{-6}
		48	1976B	<0.002	$<6.29 \times 10^{-6}$
		96	1989B	<0.002	$<6.29 \times 10^{-6}$
		168	I1016B	<0.002	$<6.29 \times 10^{-6}$
		240	I1052B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	40	24	1970C	<0.002, 0.011, 0.0062	20.1×10^{-6}
		48	1976C	<0.002	$<6.29 \times 10^{-6}$
		96	1989C	0.0035, <0.002	8.65×10^{-6}
		168	I1016C	0.0033, 0.0022	8.65×10^{-6}
		240	I1052C	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	40	24	1970D	<0.002, <0.002	$<6.29 \times 10^{-6}$
		48	1976D	(0.0016)	$<6.29 \times 10^{-6}$
		96	1989D	(0.0011), <0.002	$<6.29 \times 10^{-6}$
		168	I1016D	0.0042, 0.0024	10.4×10^{-6}
		240	I1052D	<0.002	$<6.29 \times 10^{-6}$
Control Coupon	100	24	1971A	<0.002, <0.002	$<6.29 \times 10^{-6}$
		48	1977A	<0.002	$<6.29 \times 10^{-6}$
		96	1990A	(0.0016), <0.002	$<6.29 \times 10^{-6}$
		168	I1017A	<0.002	$<6.29 \times 10^{-6}$
		240	I1053A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	100	24	1971B	<0.002, 0.0045, (0.0016)	8.89×10^{-6}
		48	1977B	<0.002	$<6.29 \times 10^{-6}$
		96	1990B	0.013, 0.0068	31.1×10^{-6}
		168	I1017B	<0.002	$<6.29 \times 10^{-6}$
		240	I1053B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	100	24	1971C	<0.002, <0.002	$<6.29 \times 10^{-6}$
		48	1977C	0.017	53.4×10^{-6}
		96	1990C	(0.0012), <0.002	$<6.29 \times 10^{-6}$
		168	I1017C	<0.002	$<6.29 \times 10^{-6}$
		240	I1053C	(0.0009), <0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	100	24	1971D	<0.002, <0.002	$<6.29 \times 10^{-6}$
		48	1977D	<0.002	$<6.29 \times 10^{-6}$
		96	1990D	(0.0008), <0.002	$<6.29 \times 10^{-6}$
		168	I1017D	(0.0012), <0.002	$<6.29 \times 10^{-6}$
		240	I1053D	<0.002	$<6.29 \times 10^{-6}$

[1] The reported LOQ of Method 200.7 is 0.002 ppm for aqueous silver; any reported values <LOQ are listed in parentheses.

[2] For samples with silver residues <LOQ, the LOQ (0.002 ppm) was used for calculating a maximum potential migration value; an average migration value is reported for samples having repeated analyses.

TABLE C.3.4. Residue Data from Migration Testing of NanoSilva Particles in LLDPE Exposed to the Food Simulant of 10 % Sodium Phosphate.

Test Sample	Exposure Temperature (°C)	Exposure Duration (hours)	Sample ID	Silver concentration in Leachate (ppm) [1]	Migration Value [2] (mg/in^2)
Control Coupon	40	24	1972A	(0.0011), 0.022	37.7×10^{-6}
		48	1978A	0.0022	6.91×10^{-6}
		96	1991A	0.0030, <0.002	7.86×10^{-6}
		168	I1018A	(0.0017), <0.002	$<6.29 \times 10^{-6}$
		240	I1054A	0.002, <0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	40	24	1972B	(0.0014), 0.0060	12.6×10^{-6}
		48	1978B	(0.0017)	$<6.29 \times 10^{-6}$
		96	1991B	0.0028, <0.002	7.54×10^{-6}
		168	I1018B	0.0030, <0.002	7.86×10^{-6}
		240	I1054B	0.0024, <0.002	6.92×10^{-6}
5.0% Coupon	40	24	1972C	(0.0013), 0.0020	$<6.29 \times 10^{-6}$
		48	1978C	0.0022	6.91×10^{-6}
		96	1991C	0.0045, <0.002	10.2×10^{-6}
		168	I1018C	0.0025, <0.002	7.07×10^{-6}
		240	I1054C	0.0022, <0.002	6.60×10^{-6}
10.0% Coupon	40	24	1972D	0.0099, 0.0088	29.4×10^{-6}
		48	1978D	0.0024	7.54×10^{-6}
		96	1991D	0.0027, <0.002	7.39×10^{-6}
		168	I1018D	(0.0017), <0.002	$<6.29 \times 10^{-6}$
		240	I1054D	0.0024, <0.002	6.92×10^{-6}
Control Coupon [3]	100	24	1973A	0.0030, 0.0028	8.81×10^{-6}
		48	1979A	0.0021	6.60×10^{-6}
		96	1992A	0.0040, <0.002	9.43×10^{-6}
		168	I1019A	0.0035, <0.002	8.65×10^{-6}
		240	I1055A	0.0020, <0.002	6.29×10^{-6}
2.50% Coupon [3]	100	24	1973B	0.0022, 0.0025	7.39×10^{-6}
		48	1979B	0.0031	9.75×10^{-6}
		96	1992B	0.0036, <0.002	8.81×10^{-6}
		168	I1019B	0.0020, <0.002	6.29×10^{-6}
		240	I1055B	0.0023, <0.002	6.76×10^{-6}
5.0% Coupon [3]	100	24	1973C	0.0021, (0.0011)	6.45×10^{-6}
		48	1979C	0.0021	6.60×10^{-6}
		96	1992C	0.0032, <0.002	8.18×10^{-6}
		168	I1019C	0.0034, 0.0032	10.4×10^{-6}
		240	I1055C	0.14, 0.10	377×10^{-6}
10.0% Coupon [3]	100	24	1973D	0.0024, 0.0027	8.02×10^{-6}
		48	1979D	0.0027	8.49×10^{-6}
		96	1992D	0.0033, <0.002	8.33×10^{-6}
		168	I1019D	0.0043, 0.0024	10.5×10^{-6}
		240	I1055D	0.88, 0.57	2280×10^{-6}

[1] The reported LOQ of Method 200.7 is 0.002 ppm for aqueous silver; any reported values <LOQ are listed in parentheses.

[2] For samples with silver residues <LOQ, the LOQ (0.002 ppm) was used for calculating a maximum potential migration value; an average migration value is reported for samples having repeated analyses.

[3] A third set of "retest" values were also reported for each coupon exposed at 100 °C at each interval. However, the sample ID numbers indicate that the samples were from a different test. As no explanation was provided, these values were not included.

p. 118 states LOQ of 0.240 ICP-MS 200.8

TABLE C.3.5. Residue Data from Migration Testing of NanoSilva Particles in LLDPE Exposed to the Food Simulant of 10 % olive Oil.

Test Sample	Exposure Temperature (°C)	Exposure Duration (hours)	Sample ID	Silver concentration in Leachate (ppm) [1]	Migration Value [2] (mg/in^2)
Control Coupon	40	24	I1211A	<0.002	$<6.29 \times 10^{-6}$
		48	I1219A	<0.002	$<6.29 \times 10^{-6}$
		96	I1227A	<0.002	$<6.29 \times 10^{-6}$
		168	I1234A	<0.002	$<6.29 \times 10^{-6}$
		240	I1258A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	40	24	I1211B	<0.002	$<6.29 \times 10^{-6}$
		48	I1219B	<0.002	$<6.29 \times 10^{-6}$
		96	I1227B	<0.002	$<6.29 \times 10^{-6}$
		168	I1234B	<0.002	$<6.29 \times 10^{-6}$
		240	I1258B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	40	24	I1211C	<0.002	$<6.29 \times 10^{-6}$
		48	I1219C	<0.002	$<6.29 \times 10^{-6}$
		96	I1227C	<0.002	$<6.29 \times 10^{-6}$
		168	I1234C	<0.002	$<6.29 \times 10^{-6}$
		240	I1258C	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	40	24	I1211D	<0.002	$<6.29 \times 10^{-6}$
		48	I1219D	<0.002	$<6.29 \times 10^{-6}$
		96	I1227D	<0.002	$<6.29 \times 10^{-6}$
		168	I1234D	<0.002	$<6.29 \times 10^{-6}$
		240	I1258D	<0.002	$<6.29 \times 10^{-6}$
Control Coupon	100	24	I1212A	<0.002	$<6.29 \times 10^{-6}$
		48	I1220A	<0.002	$<6.29 \times 10^{-6}$
		96	I1228A	<0.002	$<6.29 \times 10^{-6}$
		168	I1235A	<0.002	$<6.29 \times 10^{-6}$
		240	I1259A	(0.001)	$<6.29 \times 10^{-6}$
2.50% Coupon	100	24	I1212B	<0.002	$<6.29 \times 10^{-6}$
		48	I1220B	<0.002	$<6.29 \times 10^{-6}$
		96	I1228B	<0.002	$<6.29 \times 10^{-6}$
		168	I1235B	<0.002	$<6.29 \times 10^{-6}$
		240	I1259B	0.021	65.9×10^{-6}
5.0% Coupon	100	24	I1212C	<0.002	$<6.29 \times 10^{-6}$
		48	I1220C	<0.002	$<6.29 \times 10^{-6}$
		96	I1228C	<0.002	$<6.29 \times 10^{-6}$
		168	I1235C	<0.002	$<6.29 \times 10^{-6}$
		240	I1259C	(0.0008)	$<6.29 \times 10^{-6}$
10.0% Coupon	100	24	I1212D	<0.002	$<6.29 \times 10^{-6}$
		48	I1220D	<0.002	$<6.29 \times 10^{-6}$
		96	I1228D	<0.002	$<6.29 \times 10^{-6}$
		168	I1235D	<0.002	$<6.29 \times 10^{-6}$
		240	I1259D	(0.0008)	$<6.29 \times 10^{-6}$

1

[1] The reported LOQ of Method 200.7 is 0.002 ppm for aqueous silver; any reported values <LOQ are listed in parentheses.

[2] For samples with silver residues <LOQ, the LOQ (0.002 ppm) was used for calculating a maximum potential migration value.

TABLE C.3.6. Residue Data from Migration Testing of NanoSilva Particles in LLDPE Exposed to the Food Simulant of 10 % Sodium Phosphate and 10 % Sugar.					
Test Sample	Exposure Temperature (°C)	Exposure Duration (hours)	Sample ID	Silver concentration in Leachate (ppm) [1]	Migration Value [2] (mg/in^2)
Control Coupon	40	24	I1086A	<0.002	$<6.29 \times 10^{-6}$
		48	I1109A	<0.002	$<6.29 \times 10^{-6}$
		96	I1115A	<0.002	$<6.29 \times 10^{-6}$
		168	I1122A	0.014	44.0×10^{-6}
		240	I1166A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	40	24	I1086D	<0.002	$<6.29 \times 10^{-6}$
		48	I1109D	<0.002	$<6.29 \times 10^{-6}$
		96	I1115D	<0.002	$<6.29 \times 10^{-6}$
		168	I1122D	0.0035	11.0×10^{-6}
		240	I1166D	0.0036	11.3×10^{-6}
5.0% Coupon	40	24	I1086D	<0.002	$<6.29 \times 10^{-6}$
		48	I1109D	<0.002	$<6.29 \times 10^{-6}$
		96	I1115D	<0.002	$<6.29 \times 10^{-6}$
		168	I1122D	<0.002	$<6.29 \times 10^{-6}$
		240	I1166D	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	40	24	I1086D	<0.002	$<6.29 \times 10^{-6}$
		48	I1109D	<0.002	$<6.29 \times 10^{-6}$
		96	I1115D	<0.002	$<6.29 \times 10^{-6}$
		168	I1122D	<0.002	$<6.29 \times 10^{-6}$
		240	I1166D	<0.002	$<6.29 \times 10^{-6}$
Control Coupon	100	24	I1087A	<0.002	$<6.29 \times 10^{-6}$
		48	I1110A	0.0021	6.59×10^{-6}
		96	I1116A	0.0058	18.2×10^{-6}
		168	I1123A	<0.002	$<6.29 \times 10^{-6}$
		240	I1167A	0.0029	9.11×10^{-6}
2.50% Coupon	100	24	I1087B	<0.002	$<6.29 \times 10^{-6}$
		48	I1110B	<0.002	$<6.29 \times 10^{-6}$
		96	I1116B	<0.002	$<6.29 \times 10^{-6}$
		168	I1123B	<0.002	$<6.29 \times 10^{-6}$
		240	I1167B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	100	24	I1087C	<0.002	$<6.29 \times 10^{-6}$
		48	I1110C	<0.002	$<6.29 \times 10^{-6}$
		96	I1116C	<0.002	$<6.29 \times 10^{-6}$
		168	I1123C	<0.002	$<6.29 \times 10^{-6}$
		240	I1167C	0.0040	12.6×10^{-6}
10.0% Coupon	100	24	I1087D	<0.002	$<6.29 \times 10^{-6}$
		48	I1110D	<0.002	$<6.29 \times 10^{-6}$
		96	I1116D	<0.002	$<6.29 \times 10^{-6}$
		168	I1123D	<0.002	$<6.29 \times 10^{-6}$
		240	I1167D	0.0040	12.6×10^{-6}

1

[1] The reported LOQ of Method 200.7 is 0.002 ppm for aqueous silver; any reported values <LOQ are listed in parentheses.

[2] For samples with silver residues <LOQ, the LOQ (0.002 ppm) was used for calculating a maximum potential migration value.

TABLE C.3.7. Residue Data from Migration Testing of NanoSilva Particles in LLDPE Exposed to the Food Simulant of 10 % sodium Phosphate and 10% Sugar, with physical abrasion.					
Test Sample	Exposure Temperature (°C)	Exposure Duration (hours)	Sample ID	Silver concentration in Leachate (ppm) [1]	Migration Value [2] (mg/in^2)
Control Coupon	40	24	I1088A	<0.002	$<6.29 \times 10^{-6}$
		48	I1111A	<0.002	$<6.29 \times 10^{-6}$
		96	I1117A	0.0022	6.91×10^{-6}
		168	I1124A	<0.002	$<6.29 \times 10^{-6}$
		240	I1168A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	40	24	I1088B	<0.002	$<6.29 \times 10^{-6}$
		48	I1111B	<0.002	$<6.29 \times 10^{-6}$
		96	I1117B	<0.002	$<6.29 \times 10^{-6}$
		168	I1124B	<0.002	$<6.29 \times 10^{-6}$
		240	I1168B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	40	24	I1088C	0.0029	9.11×10^{-6}
		48	I1111C	0.027	84.8×10^{-6}
		96	I1117C	<0.002	$<6.29 \times 10^{-6}$
		168	I1124C	0.0049	15.4×10^{-6}
		240	I1168C	0.0038	11.9×10^{-6}
10.0% Coupon	40	24	I1088D	0.0043	13.5×10^{-6}
		48	I1111D	<0.002	$<6.29 \times 10^{-6}$
		96	I1117D	<0.002	$<6.29 \times 10^{-6}$
		168	I1124D	0.018	56.5×10^{-6}
		240	I1168D	0.024	75.4×10^{-6}
Control Coupon	100	24	I1089A	0.0049	15.4×10^{-6}
		48	I1112A	0.0027	8.49×10^{-6}
		96	I1118A	<0.002	$<6.29 \times 10^{-6}$
		168	I1125A	<0.002	$<6.29 \times 10^{-6}$
		240	I1169A	<0.002	$<6.29 \times 10^{-6}$
2.50% Coupon	100	24	I1089B	<0.002	$<6.29 \times 10^{-6}$
		48	I1112B	0.0024	7.54×10^{-6}
		96	I1118B	0.0062	19.5×10^{-6}
		168	I1125B	<0.002	$<6.29 \times 10^{-6}$
		240	I1169B	<0.002	$<6.29 \times 10^{-6}$
5.0% Coupon	100	24	I1089C	<0.002	$<6.29 \times 10^{-6}$
		48	I1112C	<0.002	$<6.29 \times 10^{-6}$
		96	I1118C	<0.002	$<6.29 \times 10^{-6}$
		168	I1125C	<0.002	$<6.29 \times 10^{-6}$
		240	I1169C	<0.002	$<6.29 \times 10^{-6}$
10.0% Coupon	100	24	I1089D	<0.002	$<6.29 \times 10^{-6}$
		48	I1112D	0.0034	10.7×10^{-6}
		96	I1118D	0.0024	7.54×10^{-6}
		168	I1125D	<0.002	$<6.29 \times 10^{-6}$
		240	I1169D	<0.002	$<6.29 \times 10^{-6}$

[1] The reported LOQ of Method 200.7 is 0.002 ppm for aqueous silver; any reported values <LOQ are listed in parentheses.

[2] For samples with silver residues <LOQ, the LOQ (0.002 ppm) was used for calculating a maximum potential migration value.

D. CONCLUSIONS

The submitted non-guideline study on the leaching of silver from an LLDPE polymer containing various concentrations of the Nanosilva antimicrobial colloid is not adequate and can not be graded. The study contained numerous minor and major deficiencies which are noted in the preceding section as well as cited below in Section E. A new leaching/migration study should be conducted on Nanosilva to support its use in food contact substances.

Prior to conducting a new study, the petitioner should submit a study protocol to the Antimicrobial Division for evaluation. Any protocol should clearly state the concentration of Nanosilva in the test polymer, both in terms of % silver and % nano-silver-silica particles; and adequate experimental data should be provided supporting the reported content of Nanosilva in the polymer. In addition, any analytical procedures and method should be adequately validated in conjunction with the analysis of the food simulant samples.

Although the current study is inadequate, the available data suggest that the migration of silver from an LLDPE polymer containing the Nanosilva antimicrobial colloid is low, on the order of 7-50 ng of silver/in^2 at 40°C and 100°C over periods of 24 to 240 hours.

E. STUDY DEFICIENCIES

A number of minor and major deficiencies were note in the study relating to the description of the test materials; the study design; the analytical method; and general reporting of the data.

Description of test materials:

1) The actual concentrations of silver and Nanosilva in the plastic test polymers is unclear. The beginning of the report states that the three concentrations of the colloid in the test polymers are 0.00125%, 0.0025% and 0.005%. However, based on the manufacturing information in Addendum 7 of the study report, the level of Nanosilva in the three types of coupon would be 0.125%, 0.25% and 0.5%. Finally, the experimentally determined concentrations of the colloid in the three polymers tested (based on silver content) was 0.005%, 0.034%, and 0.059%.

2) In the procedures describing the production of the "master batch", it is unclear exactly how much Nanosilva colloid was added to the plastic resin.

3) Based on the experimental determinations of their silver content, the colloid concentration of the "2.5%" plastic coupons was more than 3x below the target concentration.

Study design:

4) For each type of plastic coupon, only a single sample was collected at each sampling interval for each combination of food simulant and exposure temperature. FDA methodology recommends that the exposure tests be conducted in triplicate.

5) Only 44 plastic coupons (presumably 11 per Nanosilva concentration) were utilized for the entire study; therefore, plastic coupons were reused for different treatments. This in itself produces an uncertainty in data interpretations. Morevoer, no information was provided on tracking of which coupons were used in each treatment.

6) The volume of the food simulants used for testing (50 mL) was lower than recommended by FDA guidance. The recommended volume is 10 mL/in^2, or ~160 mL in the case of the current study. No explanation was provided as to why the 50 mL volume was selected.

Analytical method:

7) The experimental procedures used for sample preparation and analysis were not adequately validated using control samples of each food simulate fortified with silver at the end of the exposure period at fortification levels covering the range of silver concentrations expected in the test samples. Method validation procedures outlined in FDA guidance and EPA Method 200.7 were not followed.

8) Standard curves for instrument calibration were not provided from any of the analytical laboratories.

9) No information was provided supporting the various LOQs (MDLs) for silver reported by the various analytical laboratories.

10) Selected samples from several exposure tests were reanalyzed without explanation, and there was no discussion regarding why the samples were retested or about the differences in residue values between the repeated analyses.

11) For the control samples that were fortified with silver prior to exposure, no explanation was provided for the low recoveries of silver obtained from most of the food simulants.

General data reporting:

12) The entire study report lacked a clear and cohesive format. The main section of the study report (pages 10-40) is a hybrid between a protocol and an actual study description. Although study results were presented and summarized in an

acceptable tabular format, the report provided no discussion or conclusions regarding the study results.

13) The conditions and durations of sample storage were not provided

14) There was inconsistent and improper use of significant figures throughout the study in reporting data.

15) In summarizing the results, migration values were inappropriately average across different expose intervals, and duplicate analyses of individual samples were inappropriately treated as separate samples for purposes of averaging. In addition, samples in non-detectable concentrations of silver were treated as "zero values" rather that using the reported limit of quantitation.

16) However, the most critical flaws in the current study is the inadequate characterization of the test materials, nature of the leachates (silver that leaches out is it just silver ions, or nanosilver, and what is the size of it, and or if a whole silver-silicon to sulfur composite leaches out). Techniques used to estimate the quantities determine the total amount of silver and not how much silver is nano. This study totally lack in the characterization determination of nanolsilver, when present in the composite, when it is leached out, and what is the nature of the leachate silver at various pHs, any changes in the 10% sugar, salt or both etc. We believe that in various food simulants (salt, sugar, olive oil, alcohol), if the silver leaches out, its characteristics will be different the one present in the nanocomposite coupons.

F. RECOMMENDATIONS

Under the conditions and parameters used in the study, the non-guideline leaching/migration study the Agency has determined that this study is **unacceptable**. The study contained major deficiencies including: inadequate characterization of the test materials (characteristics of nanoscaled silver when it leaches); the lack of replication in the exposure tests; and the lack of adequate method validation data.

The Agency recommends that the registrants submit new protocols to run a new study, get the protocols approved by the Agency before conducting the study. The protocols can be developed based on removing the deficiencies the Agency has noted in the present review. The analytical methods to be sued for the new study should be able to identify the characteristics of nanocomposite, silver that is being leached out and other moieties found during the study

In addition, we recommend that the registrants must take into account the deficiencies noted in the DER on Product Chemistry data of Nanosilva (Memo by Earl Goad)

G. REFERENCES

EPA Method 200.7 Trace Elements in Water, Solids and Biosolids by Inductively Coupled Plasma-Atomic Emission Spectrometry. Revision 5.0, January 2001. EPA-821-R-01-101

Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations. U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition. December 2007.

The Following Appendix includes information which is classified as CBI under FIFRA Section 10(c).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Tuesday, November 03, 2009

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No.: 84610-E
DP Barcode: D370736
Product Name: Nanosilva™

From: Ian Blackwell, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

Through: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

To: Marshall Swindell, PM 33/ Demson Fuller
Regulatory Management Branch
Antimicrobials Division (7510P)

Applicant: Nanosilva, LLC

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
Covalently bound elemental silver	1.00
Other Ingredient(s):	99.00
Total:	100.00

I BACKGROUND: Nanosilva, LLC, has submitted a complete set of six acute toxicity studies to support the data requirements of their pending product, "Nanosilva™ Antimicrobial". Eurofins | Product Safety Laboratories conducted these studies.

II RECOMMENDATIONS:

1. Each of the six submitted studies is acceptable.

The acute toxicity profile for File Symbol 84610-E is currently:

Study	MRID Number	Toxicity Category	Study Status
Acute Oral Toxicity	478289-18	IV	Acceptable
Acute Dermal Toxicity	478289-19	IV	Acceptable
Acute Inhalation Toxicity	478289-20	IV	Acceptable
Primary Eye Irritation	478289-21	III	Acceptable
Primary Skin Irritation	478289-22	IV	Acceptable
Dermal Sensitization	478289-23	Nonsensitizer	Acceptable

III LABELING:

1. The signal word is "Caution".

2. The Precautionary Statements must state:

   "Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the restroom. "

3. The First Aid statements must state:

   If in Eyes:
   - Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
   - Call a Poison Control Center or doctor for treatment advice.

   The label submitted for Nanosilva also contains First Aid statements for oral ("if swallowed") and dermal ("if on skin") exposures. Based upon the acute toxicity studies assessed in this review, the EPA does not consider either of these two statements to be mandatory. However, the registrant may retain either or both of these statements if they feel the need to do so.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1, 870.1100)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 478289-18 **Study Completion Date:** 9/11/2007
Lab Study No.: 22492

Testing Laboratory: Eurofins | Product Safety Laboratories
Authors: Carolyn Lowe, LATG

Quality Assurance (40 CFR §160.12): Included

Test Material: Nanosilva™ Antimicrobial, Lot #K070605A-3810, "Tan opaque liquid"

Species: Sprague-Dawley derived albino rats
Weight: 206-220 grams **Age:** 10 weeks
Source: Ace Animals, Inc.

Conclusion:

1. **LD_{50} (mg/kg):** **Males=** (Not tested)
Females> 5,000 mg/kg
Combined= (Not tested)

2. **The estimated LD_{50} is greater than 5,000 mg/kg of body weight (b.w.).**
3. **Tox. Category:** IV **Classification:** Acceptable

Procedure (Deviations from §81-1): None

Results:

Dosage (mg/kg)	(Number Deaths/Number Tested)		
	Males	Females	Combined
5,000	(not tested)	0/3	n/a

Observations: Active and healthy.

Gross Necropsy: The lab observed no gross abnormalities.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2, 870.1200)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 478289-19 **Study Completion Date:** 9/11/2007
Lab Study No.: 22493

Testing Laboratory: Eurofins | Product Safety Laboratories
Author: Carolyn Lowe, LATG

Quality Assurance (40 CFR §160.12): Included

Test Material: Nanosilva™ Antimicrobial, Lot #K070605A-3810, "Tan opaque liquid"

Species: Sprague-Dawley derived albino rats
Weight: Males = 289-327 g, Females= 195-222 g **Age:** 9-10 weeks
Source: Ace Animals, Inc.

Summary:

1. **LD_{50} (mg/kg):**
   Males > 5,000 mg/kg
   Females > 5,000 mg/kg
   Combined > 5,000 mg/kg

2. **The estimated LD_{50} is greater than 5,000 mg/kg b.w.**

3. **Tox. Category:** IV **Classification:** Acceptable

Procedure (Deviation From §81-2): None

Results:

Reported Mortality

DOSAGE (mg/kg)	**(NUMBER DEATHS/NUMBER TESTED)**		
	Males	**Females**	**Combined**
5,000	0/5	0/5	0/10

Observations: Erythema and edema.

Gross Necropsy Findings: No gross abnormalities.

DATA REVIEW FOR ACUTE INHALATION TOXICITY (§81-3, 870.1300)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 478289-20 **Study Completion Date:** 9/11/2007
Lab Study No.: 22494

Testing Laboratory: Eurofins | Product Safety Laboratories
Author: Carolyn Lowe, LATG

Quality Assurance (40 CFR §160.12): Included

Test Material: Nanosilva™ Antimicrobial, Lot #K070605A-3810, "Tan opaque liquid"
Concentration: gravimetric = 2.05 mg/L

Species: Sprague-Dawley derived albino rat
Weight: Males= 274-315 g Females= 161-197 g
Age: 9-10 weeks
Source: Ace Animals, Inc.

Summary:

1. **LC_{50} (mg/L)** **Males** > 2.05 mg/L
   Females > 2.05 mg/L
   Combined > 2.05 mg/L
2. **The estimated LC_{50} is** > 2.05 mg/L of air.
3. **MMAD:** 2.8 **µm**
4. **Toxicity Category:** IV **Classification:** Acceptable

Procedure (Deviation From §81-3):

Results:

Reported Mortality

Exposure Concentration	(NUMBER DEATHS/NUMBER TESTED)		
	Males	Females	Combined
2.05 mg/L	0/5	0/5	0/10

Chamber Atmosphere			
Dose Level	MMAD	GSD	Particles < 4.7 µm
2.07 mg/L	2.8 µm	2.265 µm	78.2%

Chamber Environment	
Chamber Volume	6.7 liters
Airflow	31.3 – 31.5 LPM
Temperature	23-24 ° C
Relative Humidity	61-75%

Clinical Observations: Active and healthy

Gross Necropsy Findings: No gross abnormalities

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (§81-4, 870.2400)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 478289-21 **Study Completion Date:** 9/11/2007
Lab Study No.: 22495

Testing Laboratory: Eurofins | Product Safety Laboratories
Author(s): Carolyn Lowe, LATG

Quality Assurance (40 CFR §160.12): Included

Test Material: Nanosilva™ Antimicrobial, Lot #K070605A-3810, "Tan opaque liquid"
Dosage: 0.1 mL

Species: New Zealand albino rabbit **Sex:** 3 females
Weight: Not reported **Age:** "young adult"
Source: Robinson Services, Inc.

Summary:

1. **Toxicity Category:** III

2. **Classification:** Acceptable

Procedure (Deviations From §81-4): None

Results:

Observations	(number "positive"/number tested)							
	Hour	Days						
	1	1	2	3	4	7	14	21
Corneal Opacity	1/3	0/3	0/3	0/3	---	---	---	---
Iritis	3/3	3/3	3/3	0/3	---	---	---	---
Conjunctivae								
Redness	3/3	3/3	0/3	0/3	---	---	---	---
Chemosis	0/3	0/3	0/3	0/3	---	---	---	---
Discharge	3/3	1/3	0/3	0/3	---	---	---	---

- - - = no observations at this point

DATA REVIEW FOR SKIN IRRITATION TESTING (§81-5, 870.2500)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 478289-22 **Study Completion Date:** 9/11/2007
Lab Study No.: 22496

Testing Laboratory: Eurofins | Product Safety Laboratories
Study Director: Carolyn Lowe, LATG

Quality Assurance (40 CFR §160.12): Included

Test Material: Nanosilva™ Antimicrobial, Lot #K070605A-3810, "Tan opaque liquid"
Dosage: 0.5 mL

Species: New Zealand White albino rabbit
Weight: Not reported **Age:** "young adult"
Source: Robinson Services, Inc.

Summary:

1. **Toxicity Category:** IV

2. **Classification:** Acceptable

Procedure (Deviations From §81-5): None

Results: One hour following exposure, 3/3 test material-exposed animals displayed very slight erythema. Twenty-four hours after exposure, 1/3 animals displayed very slight erythema. The lab reported no other irritation.

Special Comments: None

DATA REVIEW FOR DERMAL SENSITIZATION TESTING (§81-6, 870.2600)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 478289-23 **Study Completion Date:** 9/11/2007
Lab Study No.: 22497
Testing Laboratory: Eurofins | Product Safety Laboratories
Author: Carolyn Lowe, LATG

Quality Assurance (40 CFR §160.12): Included

Test Material: Nanosilva™ Antimicrobial, Lot #K070605A-3810, "Tan opaque liquid"
Positive Control Material: Hexylcinnamaldehyde Technical (HCA)

Species: Hartley albino guinea pigs
Weight: 346-398 grams **Age:** "young adult"
Source: Elm Hill Breeding Labs

Method: Buehler Method

Summary:

1. **This Product is not a dermal sensitizer.**

2. **Classification:** Acceptable

Procedure (Deviation From §81-6): None

Procedure:
Induction Phase: Once each week for three weeks, four-tenths of a milliliter of the undiluted test substance was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system.

Challenge Phase: Twenty-eight days after the first induction dose, four tenths of a milliliter of a 75% w/w mixture of the test substance in distilled water (HNIC) was applied to a naïve site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the challenge application according to the scoring system. In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical environmental conditions and

were treated with the HNIC of the test substance at challenge only. These animals constituted the "naïve control" group.

Results:

Induction Phase (100% Test Substance): "Very faint to faint erythema (grade 0.5 – 1) was noted in all test sites during the induction phase."

Challenge Phase (75% Test Substance): "Very faint erythema (0.5) was noted in 13/20 test material-induced animals 24 hours after challenge. Similar irritation persisted in 7/20 test material-induced animals 48 hours after challenge."

Naïve Control Animals: "Very faint erythema (0.5) was noted at five of ten naïve control test sites 24 hours after challenge. Similar irritation persisted at two sites through 48 hours."

HISTORICAL POSITIVE CONTROL:

Induction Phase (HCA applied undiluted): Very faint to faint erythema (0.5-1) was noted for all positive control sites during the induction phase.

Challenge Phase (75% HCA in mineral oil): 7/10 positive control animals exhibited signs of a sensitization response (faint to moderate erythema, grade 1-2) 24 hours after challenge.

Historical Naïve Control (75% HCA in mineral oil): Very faint erythema (0.5) was noted in 1/5 naïve control animals 24 and 48 hours after challenge.

PRIA 2 – 21 Day Content Screen Review Worksheet

(EPA/OPP Use Only)

3/23/09

21 Day Screen Start Date: 8-12-09

Experts In-Processing Signature: M E HARRINGTON Date 8-18-09 Fee Paid: Yes ✓

Division management contacted on issues No_____ Yes_____ Date_____

EPA Reg. Number: 84610-E	EPA Receipt Date: 8-12-09

	Items for Review			Yes	No	N/A*
1	**Application Form** (EPA Form 8570-1)(link to form) signed & complete including package type			X		
2	**Confidential Statement of Formula** all boxes completed, form signed, and dated (EPA Form 8570-4) (Link to form)			X		
	a) All inerts (link to http://www.epa.gov/opprd001/inerts/), including fragrances, approved for the proposed uses (see Footnote A)	**yes**	**no**			
			X			
3	**Certification with Respect to Citation of Data** (EPA Form 8570-34) (Link to form) completed and signed (N/A if 100% repack)			X		
	Certificate and data matrix consistent			X		
	If applicant is relying on data that are compensable, is the offer to pay statement included. (see Footnote B)	**yes**	**no**			
	If applicable, is there a letter of Authorization for exclusive use only.					
4	**Formulator's Exemption Statement** (EPA Form 8570-27) (Link to form) completed and signed (N/A if source is unregistered or applicant owns the technical)					X
5	**Data Matrix** (EPA Form 8570-35) (Link to form) both internal and external copies (PR 98-5) (Link to PR 98-5) completed and signed (N/A if 100% repack)			X		
		yes	**no**			
	a) Selective Method (Fee category experts use)	X				
	b) Cite-All (Fee category experts use)					
	c) Applicant owns all data (Fee category experts use)					
6	**5 Copies of Label (link to http://www.epa.gov/oppfead1/labeling/lrm/) (Electronic labels on CD are encouraged and guidance is available)(link to** http://www.epa.gov/pesticides/regulating/registering/submissions/index.htm#labels **)**			X		

7	**Is the data package consistent with PR Notice 86-5** (link to PRN 86-5)	X		
8	**Notice of Filing (link to http://www.epa.gov/pesticides/regulating/tolerance_petitions.htm) included with petitions (link to http://www.epa.gov/pesticides/regulating/tolerances.htm)**			X
9	**If applicable for conventional applications, reduced risk rationale (link to http://www.epa.gov/opprd001/workplan/reducedrisk.html)**			X
10	**Required Data (link to http://www.epa.gov/pesticides/regulating/data_requirements.htm) and/or data waivers. See Footnote C.**			
	a) List study (or studies) not included with application			

Comments:

Passed S&S review MRID 478289
Could not confirm that one of the inert ingredient is approved [redacted]. Registrant was going to send a revised CSF - but has not done so as of the 15th day.

-JD

MRID 478289

* N/A – Not Applicable

Footnotes

A. During the 21 day initial content review, all CSFs will be reviewed to determine whether all inerts listed, including fragrances, are approved for the proposed uses. If an unapproved inert is identified, the applicant must either 1) resolve the inert issue by, for example, removing the inert, substituting it with an approved inert, submitting documentation that EPA approved the inert for the proposed pesticidal uses, correcting mistakes on the CSF, etc. or 2) provide the data to support OPP approval of the inert or 3) withdraw the application. Removing or substituting an inert ingredient will require a new CSF and may require submission of data. All information, forms, data and documentation resolving the inert issue must have been received by the Agency or the application withdrawn within the 21 day period, otherwise, the Agency will reject the application as described below.

To successfully complete this aspect of the 21 day initial content screen, applicants are **strongly encouraged** to verify that all inert ingredients have been approved for the application's uses **even if a product is currently registered** by consulting the inert Web

Script for Rejection Phone calls

Contact Name: Wayne Krause
Phone #: 352-615-4906
Email: wkrause@clairson.com

First Call/Initials:	**Second Call/Initials:**
Date: 8/21	**Date:** 8/24
Time: 12 PM	**Time:** 1:10 PM

This is Jennifer Drobish **, EPA contractor.**

I'm calling regarding your submission in support of
Application to Register Nanosilva Antimicrobial.

We have found the following deficiencies regarding:
PR Notice 86.5: Yes or No

Volume/Study Title: Illegible pages: 226, 304, 375, 399, 438, 520, 542, 549, 564, 852
Title: Leachability Study, Study No. 090106-1754-55-74

Volume/Study Title:

Volume/Study Title:

Additional volumes continued on back of page: Yes or No

Application Package: Yes or No
Missing Certification (selective method)
Inerts not on approved list [redacted]

These deficiencies have been approved by EPA.
The corrections can be faxed to 703-305-5060/Attn: _____.

Second Call/Email:
If we do not receive the corrections by _____, we will process your submission, accordingly. Please direct all future calls and correspondence to the appropriate EPA Risk Manager.

Inert ingredient information may be entitled to confidential treatment

Fw: Nanosilva Antimicrobial
Jennifer Drobish to: wkrause 08/24/2009 01:13 PM

Mr Krause

I have received the Certification form, thank you. All that I am waiting on now is information regarding the inert ingredients (and/or new CSF) and new copies of the illegible pages of the Leachability Study. The deadline for this application package is August 27th, please submit the information prior to that date.

Thank you
Jennifer Drobish
EPA Contractor
703-305-1671
---- Forwarded by Jennifer Drobish/DC/USEPA/US on 08/24/2009 01:10 PM ----

From:	Jennifer Drobish/DC/USEPA/US
To:	wkrause@clairson.com
Date:	08/21/2009 12:15 PM
Subject:	Nanosilva Antimicrobial

Mr Krause

This is Jennifer Drobish, EPA Contractor. I am writing to follow up on the message that I left regarding the application to register Nanosilva Antimicrobial. We have found the following deficiency regarding PR Notice 86.5:
- the Leachability Study, Study No 090106-1754-55-74 has the following illegible pages: pages 226, 304, 375, 399, 438, 520, 542, 549, 564, 852

We have also found the following deficiencies regarding the application package:
- the Certification with Respect to Citation of Data is missing (please be sure to check the "selective method" option since the company owns data)
- Inert ingredients on the CSF are not on the approved list. The CAS numbers are as follows: [redacted] If either of these ingredients is actually the active ingredient please send a new CSF indicating that. Otherwise, this can be fixed by either removing the ingredients from the CSF, replacing them with approved ingredients, submitting 100% full composition product chemistry, or by submitting information supporting the approval of the inert.

This information can either be faxed to me at 703-305-5060/Attn: Jennifer Drobish or emailed to me at this address.

Thank you
Jennifer Drobish
EPA Contractor
703-305-1671

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

August 17, 2009

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP Decision Number: D-418580
EPA File Symbol or Registration Number: 84610-E
Product Name: N ANOSILVA TM ANTIMICROBIAL
EPA Receipt Date: 12-Aug-2009
EPA Company Number: 84610
Company Name: NANOSILVA, LLC

WAYNE KRAUSE
NANOSILVA, LLC
2811 NE 14TH STREET
OCALA, FL 34470-

SUBJECT: Receipt of Registration Application Subject to Registration Service Fee

Dear Registrant:

The Office of Pesticide Programs has received your application and certification of payment. If you submitted data with this application, the results of the PRN-86-5 screen will be communicated separately. During the administrative screen, the Office of Pesticide Programs has determined that this Action is subject to a Pesticide Registration Service Fee as defined in the Pesticide Registration Improvement Act.

The Action has been identified as Action Code: A420

NEW AI;NON-FOOD USE;INDOOR FIFRA SEC 2(MM) USES;

No additional payment is due at this time.

If you have any questions, please contact the Pesticide Registration Service Fee Ombudsman at (703) 308-6432.

Sincerely,

Teresa Downs

Front End Processing Staff
Information Technology & Resources Management Division

Troy Carswell

From: paygovadmin@mail.doc.twai.gov
Sent: Monday, August 17, 2009 11:59 AM
To: Troy Carswell
Subject: Pay.Gov Payment Confirmation

THIS IS AN AUTOMATED MESSAGE. PLEASE DO NOT REPLY.

Your transaction has been successfully completed.

Payment Summary

Application Name: PRIA Service Fees
Pay.gov Tracking ID: 24VMM0HJ
Payment Agency Tracking ID: 74078421556

Name On Account: Nanotechnovation Corporation Payment Amount: $55,125.00 Payment Date: Aug 18, 2009 11:58:32 AM Account Type: Business Checking Routing Number: 063116290 Bank Account Number: XXXXX2929 Check Number: 000095 Transaction Date: Aug 17, 2009 11:58:32 AM
Decision Number:
Registration Number:

Online Payment

Step 3: Confirm Payment 1 | 2 | 3

Thank you.
Your transaction has been successfully completed.

Pay.gov Tracking Information

Application Name: PRIA Service Fees
Pay.gov Tracking ID: 24VMM0HJ
Agency Tracking ID: 74078421556
Transaction Date and Time: 08/17/2009 11:58 EDT

Payment Summary

Account Holder Name: Nanotechnovation Corporation
Payment Amount: $55,125.00
Account Type: Business Checking
Routing Number: 063116290
Account Number: *****2929
Check Number: 000095

Payment Date: 08/18/2009
Decision Number:
Registration Number:

Fee for Service

{855745C~

This package includes the following

- ◉ New Registration
- ○ Amendment

☑ Studies? ~~Fee Waiver?~~ unclear

☐ volpay ✓ % Reduction: ___

for Division

- ◉ AD
- ○ BPPD
- ○ RD

Risk Mgr. 33

Receipt No. S- 855745

EPA File Symbol/Reg. No. 84610-E

Pin-Punch Date: 8/12/2009

☐ This item is NOT subject to FFS action.

Action Code:

Requested: A420

Granted: A420

Amount Due: $ $55,125

Parent/Child Decisions:

▣ Inert Cleared for Intended Use ▣ Uncleared Inert in Product

Reviewer: MArundell Date: 8/14/09

Remarks:

NANOSILVA, LLC

August 11, 2009

Mr. Marshall Swindell
Product Manager 33
One Potomac Yard
2777 Crystal Drive
Arlington, VA 22202

Subject: New Chemical Registration (A420 PRIA)

Dear Marshall,

Nanosilva, LLC is proud to present to the U.S. Environmental Protection Agency our formal application for registration of Nanosilva™ Antimicrobial as a New Chemical (A420 Non-food use; indoor; FIFRA §2 (mm) uses). As discussed in previous meetings with members of the OPP Nanotechnology Committee, we have included in the application packet several waiver requests that address tier 1 data requirements based on product performance during an extensive Leachability Study which clearly demonstrated the reduced risk exposure profile of Nanosilva™. We appreciate your assistance and the efforts of your colleagues at the EPA during the process leading up to what we feel is a monumental step in achieving successful registration of this Nanotechnology based product. We especially would like to thank the members of the OPP Nanotechnology Committee for their support and guidance over the past 2 1/2 years and we look forward to a continued relationship as we explore potential new uses for this technology in the future.

While the registration process has been long and much more costly than we had anticipated, we feel all was justified and appropriate given the unique circumstances surrounding this application and its possible impact on future registration of Nano based technologies.

As you recall, Nanosilva™ Antimicrobial is a Silica-Sulfur-Silver Complex (colloid) based polymer additive engineered thru proprietary developments in Nanotechnology. Its intended use is for integration into polymeric intermediates with potential uses in a variety of finished treated article applications. Nanosilva, LLC will manufacture, market and distribute polymeric intermediates (Master batch) containing 5% Nanosilva™ antimicrobial additive under the brand name Polyguard™. All users will be licensed, in accordance with approved product label, for use in a specific product, within a specified field of use and under conditions consistent with regulations governing this registration.

Included in this application request, are the following documents:

- Cover Letter (including all correspondence with the EPA concerning this application)
- Transmittal Document
- EPA Application Form 8570-1

- Product Label
- Confidential Statement of Formula EPA 8570-4
- Data Matrix Table EPA Form 8570-35
- Nanosilva Antimicrobial Technical Bulletin
- Nanosilva Antimicrobial Material Safety Data Sheet
- Product Identity Studies
- Chemical Testing Studies
- Toxicology Testing Studies
- Leachability Study
- Waiver Request – did not find MS

In closing, we would like to thank you once again for your time and consideration during this registration process.

Regards,
Nanosilva, LLC

Wayne Krause
VP Operations

Attachments: Meeting Minutes and Correspondence with OPP Nanotechnology Committee

TRANSMITTAL DOCUMENT

Name and Address of Submitter:	Nanosilva, LLC 2811 NE 14th Street Ocala, Florida 34470
Regulatory action in support of which this package is submitted:	**Application for Registration of Nanosilva™ Antimicrobial [Silver – Silica Colloid – (NSPW-L30SS – Product Code)]**
EPA Reg. No./File Symbol:	No. 84610
Alternate Test Material Names:	Covalently bonded Silver-Silica Colloid in aqueous solution.
Transmittal Date:	09/20/2007

Volume No.		Administrative Materials
		Transmittal Document
1		Cover Letter
	EPA Form 8570-1	Application for Pesticide Registration (3 copies)
	EPA Form 8570-4	Confidential Statement of Formula (3 copies)
	EPA Form 8570-35	Data Matrix (3 copies)
		NANOSILVA™Antimicrobial Label (3 copies)
		NANOSILVA™Antimicrobial Technical Bulletin (3 copies)
		NANOSILVA™Antimicrobial Material Safety Data (3 copies)

Volume No.		Data Submission	MRID Number
		Product Identity	
2	830.1550	Product identity and composition	
	830.1600	Description of materials used to produce the product	
	830.1620	Description of production process	
	830.1650	Description of formulation process	
	830.1670	Discussion of formulation of impurities	
	830.1700	Preliminary analysis	
	830.1750	Certified Limits	
	830.1800	Enforcement analytical method	
		Physical/Chemical Properties	
3	830.6302	Color	
	830.6303	Physical State	
	830.6304	Odor	
	830.7220	Boiling point/boiling range	
	830.7300	Density/relative density/bulk density	
	830.7840	Water solubility: Column elution method	
	830.7000	pH	
	830.6317	Storage stability	
	830.7100	Viscosity	
	830.6320	Corrosion characteristics	
		Toxicology	
4	870.2400	Primary Eye Irritation Study in Rabbits	
	870.1200	Acute Dermal Toxicity Study in Rats	
	870.1300	Acute Inhalation Toxicity Study in Rats	
	870.1100	Acute Oral Toxicity Up and Down Procedure in Rats	
	870.2500	Primary Skin Irritation Study in Rabbits	
	870.2600	Dermal Sensitization Study in Guinea Pigs (Buehler M.)	

Company Official: Wayne Krause, Vice Pres. Operations
Company Name: Nanosilva, LLC
Company Contact: Wayne Krause, (352)-615-4906 , fax: (352)-368-1796, wkrause@clairson.com

Please read instructions on reverse before c ding form. Form Approv MB No. 2070-0060 Print Form

EPA United States Environmental Protection Agency Washington, DC 20460

[X] Registration [ ] Amendment [ ] Other

OPP Identifier Number

Application for Pesticide - Section I

1. Company/Product Number: 84610 - E

2. EPA Product Manager: Marshall Swindell

3. Proposed Classification: [X] None [ ] Restricted

4. Company/Product (Name): NANOSILVA TM, ANTIMICROBIAL

PM#: 33

5. Name and Address of Applicant *(Include ZIP Code)*:
NANOSILVA, LLC
PO BOX 5519
OCALA, FL 34478
[ ] *Check if this is a new address*

6. **Expedited Review.** In accordance with FIFRA Section 3(c)(3)(b)(i), my product is similar or identical in composition and labeling to:
EPA Reg. No. _____
Product Name _____

Section - II

[ ] Amendment - Explain below.
[ ] Resubmission in response to Agency letter dated _____
[ ] Notification - Explain below.
[ ] Final printed labels in response to Agency letter dated _____
[ ] "Me Too" Application.
[X] Other - Explain below.

Explanation: Use additional page(s) if necessary. (For section I and Section II.)

NEW PRODUCT
NEW FORMULATION

Section - III

1. Material This Product Will Be Packaged In:

Child-Resistent Packaging	Unit Packaging	Water Soluble Packaging	2. Type of Container
[ ] Yes* [X] No	[X] Yes [ ] No	[ ] Yes [X] No	[ ] Metal [X] Plastic [ ] Glass [ ] Paper [ ] Other (Specify) _____
* *Certification must be submitted*	If "Yes" Unit Packaging wgt.: 2lbs6.3oz; No. per container: 12	If "Yes" Package wgt.: ; No. per container:	

3. Location of Net Contents Information: [X] Label [ ] Container

4. Size(s) Retail Container: 1 Liter

5. Location of Label Directions: [X] On Label [ ] On Labeling accompanying product

6. Manner in Which Label is Affixed to Product: [X] Lithograph / Paper glued / Stenciled [ ] Other _____

Section - IV

1. Contact Point *(Complete items directly below for identification of individual to be contacted, if necessary, to process this application.)*

Name	Title	Telephone No. (Include Area Code)
Wayne J. Krause	President	(352) 615-4906

Certification

I certify that the statements I have made on this form and all attachments thereto are true, accurate and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

6. Date Application Received (Stamped)

2. Signature: Wayne J. Krause

3. Title: President

4. Typed Name: Wayne J. Krause

5. Date: 8-11-2009

EPA Form 8570-1 (Rev. 8-94) Previous editions are obsolete. White - EPA File Copy (original) Yellow - Applicant Copy

Form Approved OMB Nos. 2070-0060; 2070-0057; 2070-0107; 2070-0122; 2070-0164

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
1200 Pennsylvania Avenue, N.W.
WASHINGTON, D.C. 20460

Paperwork Reduction Act Notice: The public reporting burden for this collection of information is estimated to average 1.25 hours per response for registration and 0.25 hours per response for reregistration and special review activities, including time for reading the instructions and completing the necessary forms. Send comments regarding burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden to: Director, Collection Strategies Division (2822T), U.S. Environmental Protection Agency, 1200 Pennsylvania Avenue, N.W., Washington, DC 20460. Do not send the completed form to this address.

Certification with Respect to Citation of Data

Applicant's/Registrant's Name, Address, and Telephone Number NANOSILVA, LLC - 2811 NE 14TH STREET, OCALA, FL 34470 (352) 732-3244	EPA Registration Number/File Symbol 84610-E
Active Ingredient(s) and/or representative test compound(s) NANOSILVER	Date AUGUST 24, 2009
General Use Pattern(s) (list all those claimed for this product using 40 CFR Part 158) MATERIAL PRESERVATION	Product Name NANOSILVA ANTIMICROBIAL

NOTE: If your product is a 100% repackaging of another purchased EPA-registered product labeled for all the same uses on your label, you do not need to submit this form. You must submit the Formulator's Exemption Statement (EPA Form 8570-27).

☐ I am responding to a Data-Call-In Notice, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).

SECTION I: METHOD OF DATA SUPPORT (Check one method only)

☐ I am using the cite-all method of support, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).

☑ I am using the selective method of support (or cite-all option under the selective method), and have included with this form a completed list of data requirements (the Data Matrix form must be used).

SECTION II: GENERAL OFFER TO PAY

[Required if using the cite-all method or when using the cite-all option under the selective method to satisfy one or more data requirements]

☐ I hereby offer and agree to pay compensation, to other persons, with regard to the approval of this application, to the extent required by FIFRA.

SECTION III: CERTIFICATION

I certify that this application for registration, this form for reregistration, or this Data-Call-In response is supported by all data submitted or cited in the application for registration, the form for reregistration, or the Data-Call-In response. In addition, if the cite-all option or cite-all option under the selective method is indicated in Section I, this application is supported by all data in the Agency's files that (1) concern the properties or effects of this product or an identical or substantially similar product, or one or more of the ingredients in this product; and (2) is a type of data that would be required to be submitted under the data requirements in effect on the date of approval of this application if the application sought the initial registration of a product of identical or similar composition and uses .

I certify that for each exclusive use study cited in support of this registration or reregistration, that I am the original data submitter or that I have obtained the written permission of the original data submitter to cite that study.

I certify that for each study cited in support of this registration or reregistration that is not an exclusive use study, either: (a) I am the original data submitter; (b) I have obtained the permission of the original data submitter to use the study in support of this application; (c) all periods of eligibility for compensation have expired for the study; (d) the study is in the public literature; or (e) I have notified in writing the company that submitted the study and have offered (i) to pay compensation to the extent required by sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA; and (ii) to commence negotiations to determine the amount and terms of compensation, if any, to be paid for the use of the study.

I certify that in all instances where an offer of compensation is required, copies of all offers to pay compensation and evidence of their delivery in accordance with sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA are available and will be submitted to the Agency upon request. Should I fail to produce such evidence to the Agency upon request, I understand that the Agency may initiate action to deny, cancel or suspend the registration of my product in conformity with FIFRA.

I certify that the statements I have made on this form and all attachments to it are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

Signature	Date	Typed or Printed Name and Title
Wayne S. Krause	8/24/2009	WAYNE KRAUSE, VP OPERATIONS

EPA Form 8570-34 (12-2003) Electronic and Paper versions available. Submit only Paper version.

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DATA MATRIX

Date 09/20/2007	EPA Reg. No./File Symbol 84610	Page 1 of 6
Applicant's/Registrant's Name & Address Nanosilva, LLC – 2811 NE 14th Street, Ocala, Florida 34470	Product: Nanosilva™ Antimicrobial [Silver – Silica Colloid – (NSPW-L30SS – Product Code)]	
Ingredients Covalently bound Silver-Silica		

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
830.1550	Product identity and composition		Nanosilva, LLC	OWN	
830.1600	Description of materials used to produce the product		Nanosilva, LLC	OWN	
830.1620	Description of production process		Nanosilva, LLC	OWN	
830.1650	Description of formulation process		Nanosilva, LLC	OWN	
830.1670	Discussion of formulation of impurities		Nanosilva, LLC	OWN	
830.1700	Preliminary analysis		Nanosilva, LLC	OWN	
830.1750	Certified Limits		Nanosilva, LLC	OWN	
830.1800	Enforcement analytical method		Nanosilva, LLC	OWN	
830.1900	Submittal of samples	NA			
830.6302	Color		Nanosilva, LLC	OWN	
830.6303	Physical State		Nanosilva, LLC	OWN	
830.6304	Odor		Nanosilva, LLC	OWN	
830.7200	Melting point/melting range	NA			
830.7220	Boiling point/boiling range		Nanosilva, LLC	OWN	
830.7300	Density/relative density/bulk density		Nanosilva, LLC	OWN	

Signature (company representative must sign)	Name and Title	Date
Wayne Krause	Wayne Krause, Vice Pres. Operations	09/20/2007

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Ingredients Covalently bound Silver-Silica

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
830.7840	Water solubility: Column elution method		Nanosilva, LLC	OWN	
830.7860	Water solubility: generator column method	NA			
830.7850	Vapor pressure	NA			
830.7370	Dissociation constant	NA			
830.7550	Petition coefficient (*n*-octanol/water), shake flask method	NA			
830.7560	Petition coefficient (*n*-octanol/water), generator column method	NA			
830.7570	Petition coefficient (*n*-octanol/water), estimation by liquid chromatography	NA			
830.7000	pH		Nanosilva, LLC	OWN	
830.6313	Stability to normal and elevated temperature, metals and metal ions	NA			
830.6314	Oxidation/reduction: chemical incompatibility	NA			
830.6315	Flammability	NA			
830.6316	Explodability	NA			
830.6317	Storage stability		Nanosilva, LLC	OWN	
830.7100	Viscosity		Nanosilva, LLC	OWN	
830.6319	Miscibility	NA			

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Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
830.6320	Corrosion characteristics		Nanosilva, LLC	OWN	
830.6321	Dielectric breakdown voltage	NA			
830.7050	UV/visible absorption	NA			
	TOXICOLOGY				
870.2400	Primary Eye Irritation Study in Rabbits		Nanosilva, LLC	OWN	
870.1200	Acute Dermal Toxicity Study in Rats		Nanosilva, LLC	OWN	
870.1300	Acute inhalation Toxicity Study in Rats		Nanosilva, LLC	OWN	
870.1100	Acute Oral Toxicity Up and Down Procedure in Rats		Nanosilva, LLC	OWN	
870.2500	Primary Skin Irritation Study in Rabbits		Nanosilva, LLC	OWN	
870.2600	Dermal Sensitization Study in Guinea Pigs (Buehler M.)		Nanosilva, LLC	OWN	

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Wayne S. Krause	Wayne Krause, Vice Pres. Operations	09/20/2007

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[signature]	Wayne Krause, Vice Pres. Operations	09/20/2007

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			Nanosilva, LLC	OWN	

Signature (company representative must sign)	Name and Title Wayne Krause, Vice Pres. Operations	Date 09/20/2007

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			Nanosilva, LLC	OWN	

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Wayne Krause	Wayne Krause, Vice Pres. Operations	09/20/2007

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STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage and disposal

Pesticide Storage: Do not store in areas accessible to children. Keep container tightly closed. Keep container in cool area and away from direct sunlight.

Pesticide Disposal: Waste disposal must be in accordance with federal, state, and local environmental control regulations.

Container Disposal: Completely empty contents of container into processing equipment where the pesticide is used and dispose of container in sanitary landfill or by incineration, if allowable by state and local authorities.

WARRANTY STATEMENT

Nanosilva, LLC. warrants that this product conforms to the chemical description on the label. Nanosilva, LLC., makes no warranties of merchantability or fitness for a particular use or any other expressed or implied warranty except as so stated above.

NANOSILVA™ is a trademark of Nanosilva, LLC.

DATE MANUFACTURED:

LOT #

EXPIRATION DATE:

NANOSILVA™

ANTIMICROBIAL

Product Code: NSPW-L30SS

NANOSILVA™ is a covalently bound, silver-silica based antimicrobial additive engineered through proprietary developments in nanotechnology and is designed for integrated use in the manufacture of polymer, plastic and textile products.

ACTIVE INGREDIENT:

Covalently Bound Elemental Silver.....1.00%

INERT INGREDIENT:.....99.00%

TOTAL.....100.00%

KEEP OUT OF REACH OF CHILDREN

CAUTION

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS

May cause mild eye irritation. Avoid contact with eyes and skin. Wash thoroughly with soap and water after handling.

FIRST AID

IF SWALLOWED: Rinse mouth and throat thoroughly with tap water, seek medical attention.

IF IN EYES: Flush eyes with low pressure water for a least 15 minutes.

IF ON SKIN: Wash skin with soap and water, remove contaminated clothing.

EPA Registration No: XXXXX EPA Establishment No: XXXXXX

NANOSILVA, LLC.
2811 NE 14th St.
Ocala, FL 34470

Net Contents: 1 Liter (33.8 US oz)

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

NANOSILVA™ is an antimicrobial additive engineered for use in the manufacture of polymer, plastic and textile products only. It is designed for use in materials that may be integrated into products listed in the technical bulletin for NANOSILVA™ antimicrobial during the manufacturing process to impart antimicrobial activity to the manufactured product.

****See Technical Bulletin for detailed use information.****

NANOSILVA™ suppresses the growth of bacteria, algae, fungus, mold and mildew which can cause unpleasant odors, discoloration, staining and deterioration of those manufactured products.

Finished products containing NANOSILVA™ antimicrobials may not make public health claims relating to antimicrobial activity without EPA pesticide registration. When used in treated articles, this product does not protect users of any such treated article or others against food borne or disease causing bacteria, viruses or other disease causing organisms.

NANOSILVA™ antimicrobial may be integrated into materials (intermediate) that may be used in the manufacture of finished products at 5.00 ± 10% of NANOSILVA™ by weight.

AKIN GUMP
STRAUSS HAUER & FELD LLP
Attorneys at Law

CHARLES L. FRANKLIN
1.202.887.4378/fax: 1.202.887.4288
clfranklin@akingump.com

April 22, 2008

VIA OVERNIGHT DELIVERY

Ms. Betty Shackleford
Mr. Marshall Swindell
Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard (South Bldg.)
2777 Crystal Drive
Arlington, VA 22202

Re: Summary of March 31, 2008 Meeting with the OPP Nanotechnology Committee

Dear Betty and Marshall:

On Monday, March 31, 2008, representatives from NanoSilva LLC ("NanoSilva" or "Company") met with members of the Antimicrobials Division and the OPP Nanotechnology Committee ("EPA" or the "Agency") to discuss the pending registration process for NanoSilva™ Antimicrobial (the "Product"). This meeting constituted the fourth face-to-face meeting during two years of pre-application communications between the Company and the Agency regarding this silver-based, treated-article additive. The purpose was to clarify the standards and expectations EPA would apply to this Product in a formal registration application.

Overall, NanoSilva was encouraged that the Agency provided a general roadmap for the Company to follow to finalize its application. NanoSilva has complied with all of the Agency's requests for safety data and related information to date, and remains committed to providing the information necessary to support EPA's review. While the Company still questions some of the policy assumptions EPA appears to have adopted under its preliminary nanotech policy, NanoSilva intends to move forward expeditiously to complete and file the application consistent with these recommendations.

The Company also understands and appreciates the Agency's concern for due care in evaluating the first use of a registered active ingredient that meets its formal definition of a nanotech pesticide. Obviously then, NanoSilva was disappointed to learn that many of the Agency participants at the meeting had not yet reviewed the materials previously

of the Agency participants at the meeting had not yet reviewed the materials previously produced by the Company in response to your requests. In sum, the acute toxicity data and other supporting data on the physical and chemical properties demonstrate the Product's low toxicity, low concentration, and negligible likelihood of exposure or bioavailability. While much of the scrutiny on nanotechnology has focused on potential for elevated toxicity or exposure from substances available in nanoscale form, the Company expects that upon reviewing NanoSilva's information in detail, the Agency will agree that this Product exemplifies the reduced-risk characteristics that some products utilizing nanotechnology can provide.

Enclosed for your reference is a summary of the key topics discussed at the meeting along with NanoSilva's understanding of its options for moving into the formal application phase.

Sincerely,

Charles L. Franklin

Enclosure
cc: Debbie Edwards, OPP
James B. Gulliford, OPPTS

ATTACHMENT 1

SUMMARY OF MARCH 31, 2008 MEETING WITH THE OPP NANOTECHNOLOGY COMMITTEE

I. OVERVIEW

On March 31, 2008, representatives from NanoSilva LLC ("NanoSilva" or "Company") met with the representatives from the Office of Pesticide Programs ("OPP") Antimicrobials Division ("AD") and the OPP Nanotechnology Committee (collectively, the "Agency" or "EPA"). The purpose of the meeting was to discuss the Company's proposed registration application for a new treated article product using nanoscale silver as the active ingredient (Nanosilva™ or the "Product"). The meeting lasted from 2:00 p.m. to approximately 3:30 p.m. A list of attendees at the meeting is provided as Exhibit A. This summary identifies the key issues discussed and guidance offered by EPA with respect to moving forward with the review process for the proposed Product.

II. ISSUES DISCUSSED

A. Development of a Process for Moving Forward with NanoSilva's Application Process

It was important to NanoSilva that the meeting focus on providing meaningful guidance regarding EPA's expectations for NanoSilva's application. From the Company's perspective, EPA has been inconsistent in its past handling of Nanosilva™, particularly relative to other similar products, and this inconsistency and uncertainty has complicated the Company's efforts to enter into the formal registration application review process.

To illustrate, when the Company first met with EPA to discuss its proposed technology and product in January 2006, EPA staff directed the Company to prepare a "New Use" application.[1] In late 2007, however, as NanoSilva prepared to submit its New Use application, EPA informed the Company that OPP was revisiting its position, and was now considering whether the Product should be treated as a "New Chemical" based on the nanoscale size of the silver particles in the Technical Product.

The Company then met with representatives from AD and the OPP Nanotechnology Committee on November 8, 2007 ("November 8 Meeting"), both to discuss its product in greater detail and to raise concerns with EPA's evolving registration policy. EPA indicated that it had adopted an as-yet unpromulgated policy presuming that any pesticide product or ingredient meeting the Agency's definition of nanotechnology would now be regulated as a new chemical. This change in interpretation (unaccompanied by any formal rulemaking process) would mean additional data

[1] Such "new use" treatment would be consistent with the fact that multiple existing products have already been registered for use as treated-article additives using elemental silver as the active ingredient. Many of these products already release sub-nano ionic silver into the environment as a matter of course.

requirements, higher registration fees, and longer review periods than EPA had suggested during the 2006 meetings. EPA indicated that companies could make efforts to rebut the "New Chemical" presumption but provided little guidance as to what standards would apply.

Following the November 8, 2007 meeting, the Company submitted documentation establishing the foundation for "New Use" treatment of its Application and made repeated attempts for feedback on its proposal. For a variety of reasons, including the Agency's request for time to review the Company's New Use Application, a leachability study prepared by the company, and written responses to specific questions submitted by EPA staff, such feedback did not come until March 31, 2008. On March 31, 2008, however, the Parties finally met to discuss this information and the Company's proposed path to registration.

B. Clarification of Standards and Data Submission Requirements Applicable to NanoSilva™ Antimicrobial

The most significant development of the meeting was the announcement by OPP participants that, indeed, the Company should move expeditiously to file a formal registration application, albeit for a "New Chemical" registration. EPA staff identified several specific revisions to the current application approach that NanoSilva should make prior to submitting the registration application.

1. NanoSilva will apply for a "New Chemical" registration.

EPA made it clear that it is unwilling to consider NanoSilva™ Antimicrobial for treatment as a new use of silver. Noting that the bound-silver nano-particles in the Product are tightly bound in a non-nanoscale, non-leaching polymer matrix, the Company questioned whether, under EPA's interpretation of NanoSilva™, any nano-scale use of an existing registered active ingredient could ever survive EPA's "rebuttable" presumption. EPA indicated that the Agency was still developing its specific pesticide and nanotechnology policy but left open whether any specific standard or scenario would support "New Use" treatment of a nano-scale version of an active ingredient. While the Company obviously believes its product qualifies for the less onerous "New Use" registration process, the Company intends to comply with EPA's request and finalize its application in accordance with "New Chemical" registration requirements.

2. NanoSilva's registration application should address all Tier 1 Data Requirements.

OPP stated that under the "New Chemical" regulatory framework, the Company's application should address OPP's list of Tier 1 Data Requirements, either by identifying currently available data, by generating new data, or by justifying grounds for full or partial waivers. Given the widespread use of nano-silver in many other parts of the world, particularly Asia, OPP also recommended the Company explore what data might already be available with respect to nano-silver exposure and toxicity in markets where the Product is already approved and used.

3. **NanoSilva can seek waivers for certain chronic and subchronic toxicity studies if it can demonstrate negligible leaching potential from the Treated Article.**

EPA agreed that if the Company is able to demonstrate that its product poses no exposure risk once incorporated into the treated article, and demonstrates adequate worker protection precautions during the manufacturing process, the Company would be eligible to receive waivers for some or all remaining data requirements. To support such a strategy, OPP suggested that the Company conduct additional leachability testing on the Product covering representative exposure scenarios relevant to the lifecycle of the material in the context of the various uses proposed on the label.

One OPP representative suggested that the Company conduct some form of "lifecycle analysis" of the Product in light of the uses proposed in the registration application. OPP did not offer, and has not developed, specific standards for such an analysis, but suggested generally that the purpose of such an analysis would be to identify representative environmental factors that might affect the leachability characteristics of the Product. During the discussion with the parties, the following were raised as examples for consideration:

- **Choice of polymeric matrix.** OPP agreed that the Company's choice of low density polyethylene ("LDPE") constitutes a suitable "worst-case" matrix for testing the substance's leachability, given that LDPE is generally considered to be the least-stable polymer form within which the Product would be incorporated. *See, e.g.,* FDA, CFSAN, *Guidance for Industry – Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations; Final Guidance*, (Dec. 2007), *available at* http://www.cfsan.fda.gov/~dms/opa3pmnc.html#iid ("In general, under identical testing conditions, levels of migrants from low-density polyethylene (LDPE) are higher than from high-density polyethylene (HDPE) or polypropylene (PP). Migration studies done solely on LDPE (complying with 21 CFR 177.1520(a)(2)) at 100°C (approximately the highest temperature at which LDPE remains functional) are, therefore, generally sufficient to provide coverage for all polyolefins including PP, which may be used for retort applications.").

- **Extended duration.** OPP suggested that the Company conduct the leachability tests over a longer time period than conducted in the Company's initial study to determine if the leaching characteristics of the treated article change over time. EPA agreed, however, that there were reasonable limits to the length of time required for such a test. (The Company notes that under FDA's leachability guidelines for food contact substances, the maximum duration of a leachability test is 10 days. *See, e.g., id.* (Appendix II. Selected Migration Testing Protocols)).

- **Extreme temperatures.** OPP suggested that the Company conduct its leachability tests at a wider range of temperatures to determine whether a treated article may behave differently in colder or warmer temperatures.

Again, FDA has developed useful guidance on the appropriate temperature ranges to use in assessing leachability for the most critical "food contact surface" uses and these should be useful in developing NanoSilva's test methodology. *Id.*

- **Physical wear and tear.** Another parameter discussed in the meeting was testing for leaching resulting from physical wear and tear of the treated article resulting from proposed use.

- **Review of the Product using SEM or TEM technology.** OPP staff suggested that the Company may want to use SEM or TEM technology in evaluating the impact of different environmental conditions on the silver within the treated article matrix. The Company's technical experts agreed to make related inquiries.

- **Other scenarios associated with the lifecycle of proposed product uses.** In preparing its refined leachability analysis to support its Registration Application, the Company will consider the applicability of the above parameters as well as others that may prove relevant to the uses the Company elects to support in the final Application.

4. The Company can narrow the scope of EPA's data review and risk assessment by limiting the range of uses proposed in the application.

OPP commented that the Company's proposed label and technical bulletin was fairly broad, implicating a wide range of use sites and treated materials. EPA agreed that the Company could limit the range of tests, data requirements, and exposure scenarios required by identifying a more limited range of uses in its initial application.

5. EPA still needs to review the Company's acute toxicity data to assess whether the results may further mitigate the need for additional chronic and subchronic data.

The Company requested feedback from OPP regarding whether and how the Product's very favorable acute toxicity profile (as reflected in the data requested by OPP in advance of the meeting) would offset the need for additional chronic and subchronic testing. Specifically, the Company's acute toxicity testing has demonstrated that even in its most bioavailable liquid form, the Product poses minimal toxicity risk and compares favorably to the silver-based treated article additives already registered for similar uses. These data suggest that the nanoscale nature of the bound-silver silica particles used in this product does not implicate the types of unique toxicity concerns that have been identified in some other nanotechnology applications.

OPP participants acknowledged that they had not reviewed the data as intended prior to the meeting but would do so shortly thereafter, and provide relevant feedback at a later date.

C. Forms of NanoSilva™ to be Marketed

OPP requested clarification as to whether the Company intends to market the Product in its liquid form. The Company affirmed that it does not plan to sell the liquid form of the Product independently. Rather, it will custom-blend the treated-article additive into solid-plastic intermediate-master-batch pellets (in which the Product's nanoscale silver-silica clusters are bound within a *non-nanoscale* plastic matrix) before sale to outside customers.

The Company also noted that EPA had recommended that the Company test and register the colloidal form of the liquid (rather than separately registering every different form of intermediate master batch) during the Company's initial meetings with EPA in 2006. The stated rationale for registering the liquid form was to minimize the need for redundant polymer-by-polymer registrations and testing and to ensure that the acute toxicity tests focused on the most bioavailable liquid form of the Product.

III. NEXT STEPS

Based on the feedback and direction the Company received at the meeting, the Company's goal is to prepare a "New Chemical" registration application as quickly as possible so that EPA can begin its formal registration review of the NanoSilva™ product. EPA, in turn, indicated that it will review the toxicity data previously requested from the Company and provide any feedback or questions it may have associated with this data.

Exhibit A

Nano Silva Mtg 3/31/08

Marshall Swindell	USEPA	703-308-6341
[illegible]	[illegible]	[illegible]
Charles Franklin	EPA-OPP	202-~~887~~-887-4420
Wayne KRAUSE	Nanosilva EPA-OPP	352-615-4906
Mary KBrick	Nanosilva	540 338 2219
Don SAcKey	NANOSILVA	352-615-4906
Tim Dole	EPA-AD	703-305-6450
Chris Kaczmarek	EPA/VGL	202 564 3904
Matthew Crowley	EPA/HED	703 305 7606
A Najm Shamim	EPA/AD	703-308-7493
Jenny Tao	EPA/AD	703-305-2563
Elissa Reaves	EPA/HED	703-305-0312
Dennon Folk	EPA/AD	703-305-8067
Betty Shackleford	EPA/AD	(703) 308-8169
Debbie Smegel	EPA by phone	(305) 599-0199
Peter Kmiec		

CHARLES L. FRANKLIN
1.202.887.4378/fax: 1.202.887.4288
clfranklin@akingump.com

April 18, 2008

VIA HAND DELIVERY

Ms. Betty Shackleford
Mr. Marshall Swindell
Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard (South Bldg.)
2777 Crystal Drive
Arlington, VA 22202

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Overall, NanoSilva was encouraged that the Agency provided a general roadmap for the Company to follow to finalize its application. NanoSilva has complied with all of the Agency's requests for safety data and related information to date, and remains committed to providing the information necessary to support EPA's safety findings. While the Company still questions some of the policy assumptions EPA appears to have adopted in under its preliminary nanotech policy, NanoSilva intends to move forward expeditiously to complete and file the application consistent with these recommendations.

The Company also understands and appreciates the Agency's concern for due care in evaluating the first use of a registered active ingredient that meets its formal definition of a nanotech pesticide. Obviously then, NanoSilva was disappointed to learn that many of the Agency participants at the meeting had not yet reviewed the materials previously

produced by the Company in response to your requests. In sum, the acute toxicity data and other supporting data on the physical and chemical properties demonstrate the Product's low toxicity, low concentration, and negligible likelihood of exposure or bioavailability. While much of the focus for nanoscale substances has been on the potential for elevated toxicity or exposure, the Company expects that upon reviewing NanoSilva's information in detail, the Agency will agree that this Product exemplifies the potential risk *reduction* benefits that some products of nanotechnology can provide.

Enclosed for your reference is a summary of the key topics discussed at the meeting along with NanoSilva's understanding of its options for moving into the formal application phase.

Sincerely,

Charles L. Franklin

Enclosure

cc: James J. Jones, OPP
James B. Gulliford, OPPTS

ATTACHMENT 1

SUMMARY OF MARCH 31, 2008 MEETING WITH THE OPP NANOTECHNOLOGY COMMITTEE

I. OVERVIEW

On March 31, 2008, representatives from NanoSilva LLC ("NanoSilva" or "Company") met with the representatives from the Office of Pesticide Programs ("OPP") Antimicrobials Division ("AD") and the OPP Nanotechnology Committee (collectively, "the Agency" or "EPA"). The purpose of the meeting was to discuss the Company's proposed registration application for a new treated article product using nanoscale silver as the active ingredient (Nanosilva™ or "the Product"). The meeting lasted from 2:00 p.m. to approximately 3:30 p.m. A list of attendees at the meeting is provided as Exhibit A. This summary identifies the key issues discussed and guidance offered by EPA with respect moving forward with the review process for the proposed Product.

II. ISSUES DISCUSSED

A. Development of a Process for Moving Forward with NanoSilva's Application Process

It was important to NanoSilva that the meeting focus on providing meaningful guidance regarding EPA's expectations for NanoSilva's application. From the Company's perspective, EPA has been inconsistent in its past handling of Nanosilva™, particularly relative to other similar products, and this inconsistency and uncertainty has complicated the Company's efforts to enter into the formal registration review process.

To illustrate, when the Company first met with EPA to discuss its proposed technology and product in January 2006, EPA staff directed the Company to prepare a "New Use" application.[1] In late 2007, however, as NanoSilva prepared to submit its New Use application, EPA informed the Company that OPP was revisiting its position, and was now considering whether the Product should be treated as a "New Chemical" based on the nanoscale size of the silver particles in the Technical Product.

The Company then met with representatives from AD and the OPP Nanotechnology Committee on November 8, 2007 ("November 8 Meeting"), both to discuss its product in greater detail and to raise concerns with EPA's evolving registration policy. EPA indicated that it had adopted an as-yet unpromulgated policy presuming that any pesticide product or ingredient meeting the Agency's definition of nanotechnology would now be regulated as a new chemical. This change in interpretation (unaccompanied by any formal rulemaking process) would mean additional data

[1] Such "new use" treatment would be consistent with the fact that multiple existing products have already been registered for use as treated-article additives using elemental silver as the active ingredient. Many of these products already release sub-nano ionic silver into the environment as a matter of course.

requirements, higher registration fees, and longer review periods than EPA had suggested during the 2006 meetings. EPA indicated that companies could make efforts to rebut the "New Chemical" presumption but provided little guidance as to what standards would apply.

Following the November 8, 2007 meeting, the Company submitted documentation establishing the foundation for "New Use" treatment of its Application and made repeated attempts for feedback on its proposal. For a variety of reasons, including the Agency's request for time to review the Company's New Use Application, a leachability study prepared by the company, and written responses to specific questions submitted by EPA staff, such feedback did not come until March 31, 2008. On March 31, 2008, however, the Parties finally met to discuss this information and the Company's proposed path to registration.

B. Clarification of Standards and Data Submission Requirements Applicable to NanoSilva™ Antimicrobial

The most significant development of the meeting was the announcement by OPP participants that, indeed, the Company should move expeditiously to file a formal registration application, albeit for a "New Chemical" registration. EPA staff identified several specific revisions to the current application approach that NanoSilva should make prior to submitting the registration application.

1. NanoSilva will apply for a "New Chemical" registration.

EPA made it clear that it is unwilling to consider NanoSilva™ Antimicrobial for treatment as a new use of silver. Noting that the bound-silver nano-particles in the Product are tightly bound in a non-nanoscale, non-leaching polymer matrix, the Company questioned whether, under EPA's interpretation of NanoSilva™, any nano-scale use of an existing registered active ingredient could ever survive EPA's "rebuttable" presumption. EPA indicated that the Agency was still developing its specific pesticide and nanotechnology policy but left open whether any specific standard or scenario would support "New Use" treatment of a nano-scale version of an active ingredient. While the Company obviously believes its product qualifies for the less onerous "New Use" registration process, the Company intends to comply with EPA's request and finalize its application in accordance with "New Chemical" registration requirements.

2. NanoSilva's registration application should address all Tier 1 Data Requirements.

OPP stated that under the "New Chemical" regulatory framework, the Company's application should address OPP's list of Tier 1 Data Requirements, either by identifying currently available data, by generating new data, or by justifying grounds for full or partial waivers. Given the widespread use of nano-silver in many other parts of the world, particularly Asia, OPP also recommended the Company explore what data might already be available with respect to nano-silver exposure and toxicity in markets where the Product is already approved and used.

3. NanoSilva can seek waivers for certain chronic and subchronic toxicity studies if it can demonstrate negligible leaching potential from the Treated Article.

EPA agreed that if the Company is able to demonstrate that its product poses no exposure risk once incorporated into the treated article, and demonstrates adequate worker protection precautions during the manufacturing process, the Company would be eligible to receive waivers for some or all remaining data requirements. To support such a strategy, OPP suggested that the Company conduct additional leachability testing on the Product to cover other potential exposure scenarios relevant to the lifecycle of proposed uses.

One OPP representative suggested that the Company conduct some form of "lifecycle analysis" of the Product in light of the uses proposed in the registration application. OPP did not offer, and has not developed, specific standards for such an analysis, but suggested generally that the purpose of such an analysis would be to identify representative environmental factors that might affect the leachability characteristics of the Product. During the discussion with the parties, the following were raised as examples for consideration:

- **Choice of polymeric matrix.** OPP agreed that the Company's choice of low density polyethylene ("LDPE") constitutes a suitable "worst-case" matrix for testing the substance's leachability, given that LDPE is generally considered to be the least-stable polymer form within which the Product would be incorporated. *See, e.g.,* FDA, CFSAN, *Guidance for Industry – Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations; Final Guidance*, (Dec. 2007), *available at* http://www.cfsan.fda.gov/~dms/opa3pmnc.html#iid ("In general, under identical testing conditions, levels of migrants from low-density polyethylene (LDPE) are higher than from high-density polyethylene (HDPE) or polypropylene (PP). Migration studies done solely on LDPE (complying with 21 CFR 177.1520(a)(2)) at 100°C (approximately the highest temperature at which LDPE remains functional) are, therefore, generally sufficient to provide coverage for all polyolefins including PP, which may be used for retort applications.").

- **Extended duration.** OPP suggested that the Company conduct the leachability tests over a longer time period than conducted in the Company's initial study to determine if the leaching characteristics of the treated article change over time. EPA agreed, however, that there were reasonable limits to the length of time required for such a test. (The Company notes that under FDA's leachability guidelines for food contact substances, the maximum duration of a leachability test is 10 days. *See, e.g., id.* (Appendix II. Selected Migration Testing Protocols)).

- **Extreme temperatures.** OPP suggested that the Company conduct its leachability tests at a wider range of temperatures to determine whether a treated article may behave differently in colder or warmer temperatures.

Again, FDA has developed useful guidance on the appropriate temperature ranges to use in assessing leachability for the most critical "food contact surface" uses and these should be useful in developing NanoSilva's test methodology. *Id.*

- **Physical wear and tear.** Another parameter discussed in the meeting was testing for leaching resulting from physical wear and tear of the treated article resulting from proposed use.

- **Review of the Product using SEM or TEM technology.** OPP staff suggested that the Company may want to use SEM or TEM technology in evaluating the impact of different environmental conditions on the silver within the treated article matrix. The Company's technical experts agreed to make related inquiries.

- **Other scenarios associated with the lifecycle of proposed product uses.** In preparing its refined leachability analysis to support its Registration Application, the Company will consider the applicability of the above parameters as well as others that may prove relevant to the uses the Company elects to support in the final Application.

4. The Company can narrow the scope of EPA's data review and risk assessment by limiting the range of uses proposed in the application.

OPP commented that the Company's proposed label and technical bulletin was fairly broad, implicating a wide range of use sites and treated materials. EPA agreed that the Company could limit the range of tests, data requirements, and exposure scenarios required by identifying a more limited range of uses in its initial application.

5. EPA still needs to review the Company's acute toxicity data to assess whether the results may further mitigate the need for additional chronic and subchronic data.

The Company requested feedback from OPP regarding whether and how the Product's very favorable acute toxicity profile (as reflected in the data requested by OPP in advance of the meeting) would offset the need for additional chronic and subchronic testing. Specifically, the Company's acute toxicity testing has demonstrated that even in its most bioavailable liquid form, the Product poses minimal toxicity risk and compares favorably to the silver-based treated article additives already registered for similar uses. These data suggest that the nanoscale nature of the bound-silver silica particles used in this product does not implicate the types of unique toxicity concerns that have been identified in some other nanotechnology applications.

OPP participants acknowledged that they had not reviewed the data as intended prior to the meeting but would do so shortly thereafter, and provide relevant feedback at a later date.

C. Forms of NanoSilva™ to be Marketed

OPP requested clarification as to whether the Company intends to market the Product in its liquid form. The Company affirmed that it does not plan to sell the liquid form of the Product independently. Rather, it will custom-blend the treated-article additive into solid-plastic intermediate-master-batch pellets (in which the Product's nanoscale silver-silica clusters are bound within a *non-nanoscale* plastic matrix) before sale to outside customers.

The Company also noted that EPA had recommended that the Company test and register the colloidal form of the liquid (rather than separately registering every different form of intermediate master batch) during the Company's initial meetings with EPA in 2006. The stated rationale for registering the liquid form was to minimize the need for redundant polymer-by-polymer registrations and testing and to ensure that the acute toxicity tests focused on the most bioavailable liquid form of the Product.

III. NEXT STEPS

Based on the feedback and direction the Company received at the meeting, the Company's goal is to prepare a "New Chemical" registration application as quickly as possible so that EPA can begin its formal registration review of the NanoSilva™ product. EPA, in turn, indicated that it will review the toxicity data previously requested from the Company and provide any feedback or questions it may have associated with this data.

Wayne Krause

From: Franklin, Charles L. [clfranklin@AKINGUMP.com]
Sent: Friday, February 29, 2008 5:41 PM
To: PJK@kappalabs1.com; MaryBruch; Wayne Krause; Don Sauey
Subject: FW: Additional Questions for NanoSilva

Here is the follow-up list of questions compiled by EPA on our package. Notice in particular the last question seeking clarification on the mode of action (silver verses radical oxygen).

What are schedules like on Monday morning? Can we schedule a quick meeting at 11 am to discuss these questions and any further action needed on the larger package?

-----Original Message-----
From: Fuller.Demson@epamail.epa.gov
[mailto:Fuller.Demson@epamail.epa.gov]
Sent: Friday, February 29, 2008 5:22 PM
To: Franklin, Charles L.
Subject: Additional Questions for NanoSilva

Hi Charles,

Below are additional questions we would like for you to consider when we meet in the near future. These questions are in addition to the data set that we asked your group to send. If you have any questions, please feel free to contact Marshall or me.

Thanks for your patience and have a great weekend!

Demson

In determining your level of detection, you mentioned the testing protocol used was the Induction Coupled Plasma (ICP) analysis. What was analyzed (the silver ion ,nanosilver or the sliver-sulfur-silica complex)?

The Confidential Statement of Formula (CSF) was unclear leaving us unsure of what formulation you intend to market. We assume that the CSF you submitted describes the components used to formulate the silver, sulfer, silica complex as a colloid. However, in your presentation you mentioned that this colloid woud be further formulated into a polymeric matrix (i.e. a master batch). Are you intending to market Nanosilva as a colloid or as a master batch or both? The reason this information is important is to determine how we would assess risk for the polymeric meric matrix as opposed to the colliod. The way you market this product will determine what data we may ask your company to submit.

Describe the physical and chemical characteristics of the silver-sulfur-silica complex (i.e., size, shape, surface area, catalytic activity, functionalization, coating, reactive oxygen species). In addition, could you provide more information on physical and chemical characteristics of the nano silver particle itself?

Could you provide better information regarding the aggregation potential (zeta potential surface charge) this complex has under varying pH levels?

You made references to literature that was used to assess the low

toxicity of silver. Could you reference that literature (provide a bibliography).

In regards to your leaching studies, what is leaching out of the matrices (the nanosilver, sliver-sulfur-silica complex or elemental silver). What was the detection level used to determined that the compound did not leach?

The label indicates that Nanosilva may be integrated into intermediate materials at 5.00 + 10% by weight while the presentation indicates that the rate is 5% to 10%. This needs to be clarified. Also, please be prepared to describe how the proposed sites listed in your technical bulletin are to be used by the public (to assure that the use is clearly non-food as opposed to food). There were several sites we determined that may pose potential conflicts (conveyor belts, brush bristles, sponges, wiping clothes, packaging, adhesive and sealants to name a few) as it relates to sites that could be potentially exposed to food. The HVAC use should also be clarified. We would be concerned about potential inhalation exposures for this use.

A question arose concerning your handout, in particular, the 3rd paragraph on page 2 of Exhibit A " ....the covalently bonded silver contained within the plastic polymer is able to exert antimicrobial characteristics by conversion of molecular oxygen to a short-lived free radical form of Oxygen molecule at or near the polymer/air interface...." Could you describe what's going to happen next? Does the short-lived free radical form of Oxygen molecule or "the covalently bonder silver" carry out the antimicrobial function?

Demson Fuller
Chemical Review Manager
Antimicrobials Division
703-308-8062 (work)
703-308-8481 (fax)

NANOSILVA LLC

RESPONSES TO EPA QUESTIONS SUBMITTED FEBRUARY 29, 2008

1. In determining your level of detection, you mentioned the testing protocol used was the Induction Coupled Plasma (ICP) analysis. What was analyzed (the silver ion, nanosilver or the sliver-sulfur-silica complex)?

The Company conducted its testing on the silver-sulfur-silica complex at the recommendation of EPA staff, who recommended that the Company register the silver-sulfur-silica complex for subsequent blending into various polymeric intermediate master batches.

The Company conducted three forms of testing to determine the concentrations of actual silver within the silver-silica colloid form of the product.

- Induction Coupled Plasma ("ICP") analysis;
- Proton Induced X-ray Emission ("PIXE"); and
- Neutron Activation Analysis ("NAA").

The Company elected to use three separate bench methods to ensure the accuracy and robustness of its data. The measured levels of elemental silver were consistent across each bench method. For a detailed analysis of the Company's methodology in analyzing the product, please refer to the Enforcement Analytical Method Study submitted as part of Volume 1 of the draft Registration submission.

2. The Confidential Statement of Formula (CSF) was unclear leaving us unsure of what formulation you intend to market. Are you intending to market Nanosilva as a colloid or as a master batch or both?

At the recommendation of EPA staff during preliminary meetings in January and February 2007, the Company is registering the product in its silver-silica colloid formulation, with the intention of blending the colloidal formulation into various polymeric master batches before marketing and distribution for use in treated articles. The Company does not plan to market the colloidal solution alone.

3. Describe the physical and chemical characteristics of the silver-sulfur-silica complex (*i.e.*, size, shape, surface area, catalytic activity, functionalization, coating, reactive oxygen species). In addition, could you provide more information on physical and chemical characteristics of the nano silver particle itself?

With respect to the physical and chemical characteristics of the silver-sulfur-silica complex, the Company directs the Committee to Volumes I and II of the supporting data for the registration package. The Company also provides additional discussion of the complex's chemical characteristics as part of its response to question 10 below.

With respect to the chemical characteristics of nanosilver particles themselves, the Company directs EPA to . . . . [Wayne/Peter, Mary, what do we have?]

4. Could you provide better information regarding the aggregation potential (zeta potential surface charge) this complex has under varying pH levels?

The Company requests additional clarification as to what information EPA is requesting and whether such data are referenced as part of any standard testing protocols EPA typically requires for a treated article registration. In the alternative, we would be happy to discuss this question in further detail to determine what data EPA is requesting.

5. You made references to literature that was used to assess the low toxicity of silver. Could you reference that literature (provide a bibliography).

A non-exhaustive bibliography of publicly-available documents and publications discussing the low toxicity of silver is attached as Exhibit __.

6. In regards to your leaching studies, what is leaching out of the matrices (the nanosilver, sliver-sulfur-silica complex or elemental silver)? What was the detection level used to determine that the compound did not leach?

The leaching study was designed to detect the presence of leached silver in any form, at a level of detection of .02 parts per billion. For detail on the protocol used, the Company directs the Committee to the complete leaching protocol, attached as Exhibit __.

7. The label indicates that Nanosilva may be integrated into intermediate materials at 5.00 + 10% by weight while the presentation indicates that the rate is 5% to 10%. This needs to be clarified.

The label should be read to allow integration of NanoSilva into intermediate materials at concentrations between 5% and 10%. The Company will work with EPA to make the necessary adjustments to the label language to clarify this point.

8. Please be prepared to describe how the proposed sites listed in your technical bulletin are to be used by the public (to assure that the use is clearly non-food as opposed to food). There were several sites we determined that may pose potential conflicts (conveyor belts, brush bristles, sponges, wiping clothes, packaging, adhesive and sealants to name a few) as it relates to sites that could be potentially exposed to food.

The Company prepared its list of proposed use sites based on those that have already been approved for existing registered silver-based antimicrobial treated article additives. The Company would be willing to discuss specific concerns that you may have with use sites or use-site descriptions on the proposed label and technical bulletin.

9. The HVAC use should be clarified. We would be concerned about potential inhalation exposures for this use.

Again, the Company identified HVAC, along with other proposed use sites, based on the uses already approved by EPA for the currently-registered silver-based treated article additives –

all of which have a greater risk of bioavailability. Current HVAC use of silver ion technology is in flu/duct liners or on surfaces exposed to humid air in HVAC systems. The activity of Ag+ (ions) is dependent on release at the surface inhibiting accumulation of fungus on these surfaces. The contamination and distribution of fungi or bacteria into the environment has been the source of allergic responses and infection and potentially, "sick building" syndrome. The use of Nanosilva™ would be the same. There is a very low to non-existent risk from release of silver nanoparticles from surfaces of polymeric materials used in HVAC systems. Other antimicrobial chemicals used in HVAC systems (Bronopol) that have been studied for their effects in room environments have shown very low concentrations in use and have been registered. Some products registered as disinfectants have been sprayed into HVAC systems and produced inhalation risks. An incorporated material like Nanosilva™ that does not leach from the surface would not produce an inhalation risk.

The Company remains willing to discuss any concerns you have with this or other uses as part of our meeting on moving forward with the registration process.

10. A question arose concerning your handout, in particular, the 3rd paragraph on page 2 of Exhibit A "....the covalently bonded silver contained within the plastic polymer is able to exert antimicrobial characteristics by conversion of molecular oxygen to a short-lived free radical form of Oxygen molecule at or near the polymer/air interface...." Could you describe what's going to happen next? Does the short-lived free radical form of Oxygen molecule or "the covalently bonder silver" carry out the antimicrobial function?

The next step in the antimicrobial action at or near the polymer/air interface after the formation of free radicals of oxygen is the recognition, that they are transitory and highly reactive. If microbes or debris are present at the surface, the free radical will react with cell wall/cellular proteins and inactivate constituents of the cell. If not, the free radical oxygen can recombine and form molecular oxygen or be absorbed by other surface material. The silver as Ag+ is not the active moiety, but the oxygen radical is not produced without the covalently bound silver nanoparticles in the matrix of the polymer. In other technologies, the Ag+ must be released to the surface to be active as an antimicrobial.

Attached, as Exhibit __, is a published Paper that provides an overview of the use of silver as a catalyst to promote oxidation and antimicrobial mechanism. [reviewing other articles for potential inclusion as well]

AKIN GUMP
STRAUSS HAUER & FELD LLP

Attorneys at Law

CHARLES L. FRANKLIN
1.202.887.4378/fax: 1.202.887.4288
clfranklin@akingump.com

December 11, 2007

Mr. Marshall Swindell
Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard (South Bldg.)
2777 Crystal Drive
Arlington, VA 22202

Re: Registration Strategy of NanoSilva™ Antimicrobial

Dear Marshall:

Thank you for taking the time to meet with the Nanotechnology Committee on November 8, 2007. The NanoSilva team appreciates the feedback, as well as the enthusiasm you and your colleagues shared for the technology and proposed product. As you requested, enclosed please find a summary of the presentation and subsequent discussion along with supporting exhibits.

We are eager to move forward with the registration of NanoSilva™ Antimicrobial pursuant to the roadmap outlined during the meeting. As a follow-up to our meeting, we are preparing a short proposal that will provide OPP with the information necessary to make its required safety findings while allowing NanoSilva to begin commercializing this reduced-risk, treated-article product in a timely fashion.

Please do not hesitate to contact us if you have any questions or need additional information. Upon the completion of your review, we would welcome the opportunity to discuss the next steps in preparing a final registration application package.

Sincerely

Charles L. Franklin

Enclosures

SUMMARY OF NOVEMBER 8, 2007 PRESENTATION TO THE OPP NANOTECHNOLOGY COMMITTEE BY NANOSILVA LLC

I. OVERVIEW

On November 8, 2007, representatives from NanoSilva LLC ("NanoSilva" or "Company") met with the OPP Nanotechnology Committee (the "Committee") to present its registration application for a new treated article product using nanoscale silver as the active ingredient. In advance of the presentation, NanoSilva provided copies of key portions of its registration application (attached as Exhibit A) and copies of its presentation slides (attached as Exhibit B). The meeting lasted from 3:00 p.m. to approximately 5:00 p.m., including subsequent informal discussions between NanoSilva and individual Committee members.

II. ATTENDEES

A list of attendees at the meeting is provided as Exhibit C.

III. PRESENTATION SUMMARY

Mr. Marshall Swindell opened the meeting, briefly discussing his prior consultations with the Company on their tentative registration and his decision to ask the Company to formally present to the Committee. After the attendees introduced themselves, Mr. Swindell turned the floor over to NanoSilva.

After brief introductory remarks, Mr. Wayne Krause, Vice President of NanoSilva, addressed the Committee, providing background information on the Company, its overseas partners, and key members of the design team. Mr. Krause then provided a general overview of the bound-silver technology used in the active ingredient, including its unique stability, low toxicity, and low leachability, both in its colloidal form (as submitted for registration) and as incorporated into the final treated article matrix. Mr. Krause confirmed that the product qualified for treatment as a "nanotechnology" product under the standards established in the EPA Nanotechnology White Paper.

Following Mr. Krause's presentation, Dr. Peter Kmieck, Director of Kappa Laboratories, Inc., presented data and testing methodologies in support of the NanoSilva™ Antimicrobial registration application. He demonstrated the minimal toxicity, low concentration, and minimal exposure risk from the product as formulated and as used in end products. Dr. Kmieck discussed the testing that Kappa Laboratories has performed for NanoSilva on the leachability of the bound silver and the antimicrobial efficacy of the product formulated in a treated article.

D. Comparison of Concentration of Bound Nanosilver in NanoSilva™ Antimicrobial to Ionic Silver in Registered Products

As demonstrated on slide 7 of the presentation (Exhibit B at p. 7), the colloidal form of NanoSilva™ Antimicrobial contains silver at a concentration of approximately 8,000 parts per million. Once compounded into the polymeric intermediate master batch, the concentration of bound elemental silver falls to approximately 160 parts per million. Ultimately when incorporated into the polymeric matrix of the final treated article, the concentration of silver will fall to only 8 – 20 parts per million.

By comparison, the label for Agion Silver Antimicrobial AD, one of the currently registered silver-based antimicrobial products, indicates significantly higher concentrations of silver in the technical product at roughly 220,000 parts per million. See e.g., EPA, OPP, Notice of Pesticide Registration, AgION® Silver Antimicrobial Type AD Pesticide Label (Exhibit D) (describing the product as 22 percent silver). Even after incorporation into the final treated article, the label directions suggest that the concentration of silver, the active ingredient, in a final treated article would remain between 220 and 11,000 parts per million, 10 to 550 times higher than the silver concentrations in NanoSilva-treated products. Id.

E. Comparison of Bioavailability of Bound Elemental Silver with Ionic Silver

The distinction between currently-registered silver products and NanoSilva™ Antimicrobial is most notable when comparing the relative concentrations of bioavailable silver. As Dr. Kmieck and Mr. Krause explained in the presentation, the nanoscale silver clusters in NanoSilva™ Antimicrobial remain tightly bound in the treated article, thus allowing no potential of leaching, even when incorporated in the least stable plastic matrices. See Exhibit B at p. 9 (description of core technology). NanoSilva's leaching studies are consistent with this fact. See id. at p. 23 (summarizing leaching analysis). This non-leaching characteristic results from the strong nonreactive covalent bonds that hold the silver clusters to the silica-sulfur together within the polymeric matrix of the treated article.

The differing bioavailability profiles of NanoSilva and other Silver-based treated article antimicrobials are also reflected in the distinct modes of action. Currently-registered products rely on the release of silver for their antimicrobial effect. See e.g., Agion®, Technology - "How it Works" (2006) (Exhibit E).[1] NanoSilva™ Antimicrobial does not require the release of any silver into the environment to provide the antimicrobial activity to the treated article. See Exhibit B at p. 14 (describing mode of action for treated article).

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[1] Available at http://www.agion-tech.com/Technology.aspx?id=156 (last visited Nov. 28, 2007).

F. Toxicity Based on Existing Data and Low Bioavailability.

NanoSilva™ Antimicrobial's low bioavailability, when considered with the minimal toxicity indicated by NanoSilva's acute toxicity testing and the extensive body of literature available on bound-silver, indicates that the product can serve as a reduced-risk alternative to the products already being marketed for use in similar treated articles. See e.g., Exhibit B at p. 26 ("Summarizing the Results of NanoSilva's Hazards Analysis"); USGS, Mineral Commodity Profiles: Silver, Open-File Report 2004-1251 (2005) (Exhibit F) ("Most silver compounds, aside from those containing toxic anions, such as arsenate or cyanide, are essentially nontoxic. Compounds that dissociate in solution and provide significant concentrations of free silver ions can be toxic to bacteria and to freshwater aquatic organisms, but compounds and complexes in which the silver is tightly bound, such as silver sulfide and thiosulfate complexes, are innocuous."). Indeed, given the lack of mobility of the nanosilver particles contained in NanoSilva™ Antimicrobial, and subsequently treated articles, the only meaningful source of bioavailability would be intimate dermal exposure, for which NanoSilva's test have indicated low toxicity.

G. Clarification of TEM Images of NanoSilva Complex Particles

The entire magnified particle in the photo provided by Dr. Kmieck and Mr. Krause falls within the general size range of 30 to 50 nanometers. The legend is not to scale as a result of a formatting error on the slide following magnification of the particle image.

The small black dots covering the larger particle are silver clusters attached to the silica nanoparticle. Any marks surrounding the NanoSilva Complex particle likely constitute trace substances or slide contaminants.

H. NanoSilva's Intention Not to Make Food-Use Claims

The Company does not intend to seek a formal "food use" registration at this time, and will work with OPP to address and modify specific claims on its current proposed label or Technical Bulletin as necessary prior to the initial registration. OPP generally appears flexible in its labeling requirements for silver-based treated articles where, as here, the applicant can provide data to demonstrate the lack of any bioavailable silver from treated articles.

I. Confirmation of Non-leachability

Kappa Laboratories has conducted all end-use product leachability testing on Low Density Polyethylene (LDPE) coupons treated with NanoSilva™ Antimicrobial at the concentrations recommended on the label. Kappa Laboratories chose LDPE on the basis that it is the polymer matrix most likely to result in releases due to its softness and relative instability. In other words, Kappa Laboratories conducted its tests on the "worst-case" polymer matrix. In conducting the tests, Kappa Laboratories adhered to ASTM test

methods at pH levels ranging from 2.0 to 10.0. To the extent that OPP has specific concerns regarding the adequacy of existing leachability/exposure data for specific uses and/or conditions, the Company is willing to take reasonable steps to develop a protocol for further data (or modify the proposed label to address such concerns). In particular, the Company reiterates its offer to conduct additional leachability testing consistent with FDA's "food contact surface protocol" provided that the Committee would accept such data and consider it in resolving outstanding questions.

J. Form of Active Ingredient Used for Different Tests

NanoSilva conducted tests on both the technical grade colloidal material and also on the product when incorporated into a treated article Low Density Polyethylene (LDPE) polymer matrix. This was the approach recommended by OPP staff during the Company's early consultations and appears to be the same approach used by other registrants that have registered silver-based treated article products.

V. POLICY ISSUES

A. "Nano" Applications as "New Use" vs. "New Chemical"

Ms. Betty Shackelford indicated that while EPA might adopt a general policy under which pesticide products containing registered active ingredients in nanoscale sizes would be treated as "new chemicals," Companies like NanoSilva would have the opportunity to present arguments for treatment as new uses where appropriate. NanoSilva intends to provide evidence to support "new use" treatment for its specific use of silver in this product. As it will demonstrate in detail in a later submission, this "new use" approach makes particular sense in the current case where the nanoscale particles are fully bound in a non-leaching, non-nano matrix that eliminates the unique exposure and toxicity concerns that have prompted questions about nanotechnology risks. Moreover, given silver's long and extensive history as an antimicrobial product, NanoSilva can demonstrate that existing silver registrations already assess the risks of silver at sizes far smaller than that proposed for NanoSilva™ Antimicrobial, and at far greater concentrations and levels of bioavailability.

B. Alternatives to Conducting New Studies

To the extent that OPP does identify data requirements not addressed in NanoSilva's current application package, OPP staff noted that NanoSilva may be able to obtain a waiver from the typical new use testing requirement based on the presence of existing data or other product-specific factors. Specifically:

1. NanoSilva can identify existing data in the publicly-available literature that address the relevant risk issues. If the Company can demonstrate the validity of such data as applied to NanoSilva™ Antimicrobial, such data would be relevant to any request for waiver for further Toxicology testing.

2. NanoSilva can submit an argument for why specific tests should be waived based on the unique characteristics of the product. Indeed, several members of the Committee agreed that if NanoSilva's leaching data supports the lack of any exposure risk from labeled uses, OPP would be less inclined to seek additional toxicity testing.

3. NanoSilva may be able to obviate the need for specific data by modifying the proposed label to remove or narrow specific uses that might create greater risk concerns within OPP. Registration could proceed by moving forward with a narrower range of labeled uses while developing data for other uses that may raise greater concerns. Labeling should correspond with this limited set of proposed uses.

NanoSilva believes that all three of these approaches may apply in the case of the NanoSilva application and will pursue these points further in finalizing its registration package.

C. Applicability of Past Policies with Respect to Data Requirements for Silver Products

OPP staff acknowledged that some of the treated article products approved previously may have been held to a lower data standard than that being discussed with respect to NanoSilva. Apparently, since registering the dozen or so ionic silver products currently on the market, the Agency has come to believe that more data may be necessary to support these existing products going forward. Indeed, OPP is likely to seek additional data on these existing products during the Registration Review Process for silver, scheduled for FY2009. See EPA, Office of Pesticide Programs, Registration Review Schedule Summary (Nov. 13, 2006) (attached as Exhibit G).

NanoSilva is committed to working with OPP to address any outstanding risk issues raised by its specific registration application, and hopes to proceed under the current standards and then take part in the industry-wide registration review process scheduled for FY2009. This approach would ensure a level playing field and ensure that OPP's regulatory approach "enable[s] rather than hinder[s] innovation" in the field. See Memorandum from John H. Marburger, III, Director, Office of Science and Technology Policy and James L. Connaughton, Chairman, Council on Environmental Quality, to the Heads of Executive Departments and Agencies (Nov. 8, 2007) (attached as Exhibit H). The Company also suspects that, when compared side-by-side with the other silver-based products going through registration review, NanoSilva™ Antimicrobial's unique stability and nonleaching characteristics will obviate the need for many of the product-specific data requirements that OPP may identify with respect to the current list of products which rely on free ionic silver for their efficacy.

VI. PROPOSED NEXT STEPS

Drawing on the feedback from EPA at the OPP Nanotechnology Committee presentation, the Company is preparing a proposal for moving forward with a targeted registration for NanoSilva™ Antimicrobial. As the proposal will demonstrate, the

product's incorporation of nanoscale silver particles within a stable and nonleaching polymer matrix (far more stable than those silver-products already registered as treated articles) makes it an appropriate exception to any presumption OPP may adopt regarding treating "nanotech" forms of registered ingredients as new chemicals. NanoSilva's proposed registration package will address the Committee's data concerns, allow the Company to move forward toward commercialization of some, if not all, of the potential uses, and ensure a level playing field among the many silver-based products already registered. The Company welcomes any feedback that the Committee can offer regarding this strategy.

technology

How it Works

Agion's customized antimicrobial solutions incorporate silver ions in a zeolite carrier. The silver ions exchange with other positive ions (often sodium) from the moisture in the environment, effecting a release of silver "on demand".

The patented multi-faceted zeolite crystal carrier provides a three dimensional release mechanism (Figure 1) that provides efficient release of silver ions independent of particle orientation in the substrate.

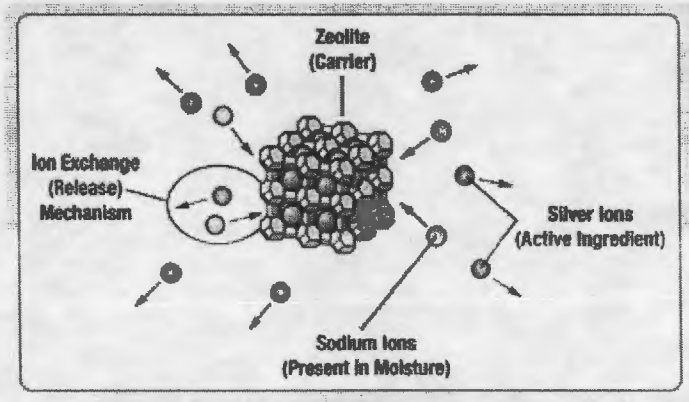

Figure 1.

The patented multi-faceted zeolite crystal carrier

Figure 2 shows the ion exchange process. Zeolite crystals containing silver ions are randomly oriented and distributed through the surface of a fiber, polymer or coating. In conditions that support bacterial growth, positive ions, in ambient moisture, exchange with silver ions at reversible bonding sites on the zeolite. The exchanged silver ions are now available to control microbial growth.

- **New to antimicrobial solutions?**
- **Get the Agion advantage!**
- **Agion customer login.**

Featured Customer

OMNIPURE FILTER COMPANY

Omnipure is a world-class vertically integrated supplier of water filtration components for industrial, commercial and residential applications. The company now features Agion antimicrobial technology in its line of carbon block water filters and patented Aquabond technology to gain better tasting and sediment free water.
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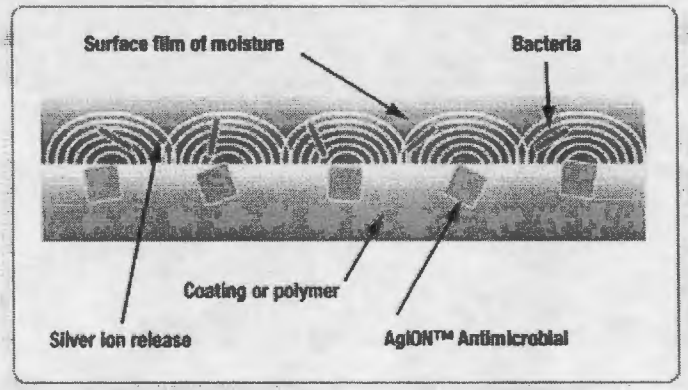

Figure 2.

The ion exchange process

Fighting microbes in three ways

Silver ions attack multiple targets in the microbe to prevent it from growing to a destructive population. This tri-modal action fights cell growth in three ways:

1. Prevents respiration by inhibiting transport functions in the cell wall
2. Inhibits cell division (reproduction)
3. Disrupts cell metabolism

Depending on the microorganism, Agion's antimicrobial technology has been shown to initially reduce microbial populate ions within minutes to hours while maintaining optimal performance for years.

60 Audubon Road, Wakefield, MA 01880 | Phone: 781-224-7100 | Fax: 781-246-3340

Exhibit F.

USGS, Mineral Commodity Profiles: Silver, Open-File Report 2004-1251 (2005).

EPA Office of Pesticide Programs: Registration Review Schedule Summary

November 13, 2006

Planned Schedule for Opening Registration Review Dockets

	FY '07 Dockets	FY '08 Dockets		FY '09 Dockets	FY '10 Dockets	
ANTIMICROBIALS						
	Benzenemethanaminium	(Coco alkyl)amine salts		Silver (and compounds)	Ethanolamine, 2-	
	Busan 1024	Bromine chloride	BR	Oxazolidine-E	2,4-Imidazolidinedione, 3-bromo-1-chloro-5,5-dimethyl-	
	2,4-Imidazolidinedione	Bromine	BR	Tris(HOCH2-)nitromethane	3H-1,2-Dithiol-3-one, 4,5-dichloro-	
	Zinc borate (3ZnO, 2B03, 3.5H2O; mw 434.66)	Inorg. halides	BR	Carbendazim	p-Chloro-m-xylenol	
		Capric acid		2-(Decylthio)ethanamine hydrochloride	Cosan 145 (*)	
		Mineral bases, strong		Barium metaborate	Dibromo-3-nitrilopropionami	
		OBPA		Mineral acids	Copper, and oxides	CU
		Biobor (*)		Peroxy cmpds	Napthenate salts	CU
Antimicrobials Totals	4	8		8	8	
		Note: BR = Bromine Compound; CU = Coppers Group				
BIOCHEMICALS						
	Linalool	Liquid Nitrogen		Wood oils and gums	Verbenone & 4-Allyl Anisole	
	Chitin	Thyme Herbs & Ground Sesame Plant		Atonik	Egg Solids	
	Farnesol & Nerolidol	L-Lactic acid		Boll weevil attractants	IBA	
		Azadirachtin		Garlic Oil	Pelargonic acid and ester	
		Dried blood		Capsaicin	Ethylene	
Biochemicals Totals	3	5		5	5	
MICROBIALS						
	Trichoderma species	Bacillus subtilis		Streptomyces griseoviridis	Candida oleophila	
	Pseudomonas syringae	Nosema locustae		Metarhizium anisopliae	Encapsulated Bt proteins	
	Pseudomonas flourescens			Ampelomyces quisqualis	Beauveria bassiana	
Microbials Totals	3	2		3	3	

Exhibit G.

EPA, Office of Pesticide Programs, Registration Review Schedule Summary (Nov. 13, 2006).

USGS
science for a changing world

MINERAL COMMODITY PROFILES

Silver

By W.C. Butterman and H.E. Hilliard

Open-File Report 2004-1251

U.S. Department of the Interior
U.S. Geological Survey

U.S. Department of the Interior
Gale A. Norton, Secretary

U.S. Geological Survey
Charles G. Groat, Director

U.S. Geological Survey, Reston, Virginia 2005

For product and ordering information:
World Wide Web: http://www.usgs.gov/pubprod
Telephone: 1-888-ASK-USGS

For more information on the USGS—the Federal source for science about the Earth,
its natural and living resources, natural hazards, and the environment:
World Wide Web: http://www.usgs.gov
Telephone: 1-888-ASK-USGS

third Idaho silver mine of long standing, the Sunshine, was closed permanently early in the year. The 32 leading domestic silver-producing mines listed by Hilliard (2002, p. 70.15) accounted for virtually all domestic production in 2001. Of these 32 mines, 2 mined silver ores, 15 mined gold ores, 7 mined copper and copper-molybdenum ores, 6 mined lead and lead-zinc ores, and 2 mined zinc ores. About 1,300 mine and mill workers were engaged in the production of domestic silver in 2001.

Silver is extracted from its ores and sometimes refined, at least partially, at base-metal smelter/refineries. At gold mines, gold-silver doré bullion is the usual product. The crude silver produced at these base-metals plants and at gold mines is usually shipped to precious-metals refineries for refining to commercial-grade silver bullion. In the United States, 22 principal refiners of commercial-grade silver operated in 2001 with an estimated output of 2,800 t of primary silver and 1,700 t of secondary silver. Most of these refineries were concentrated in the eastern and western seaboard States, which are nearer to the silver fabricating industries than to the silver mines.

Thousands of artisans and companies fabricate silver, but the bulk of silver consumption is accounted for by a relatively small number of companies. For example, in the United States, which uses about 20 percent of the world's silver, about 30 companies consume more than 90 percent of the silver. Extrapolation of those figures suggests that perhaps 100 to 150 large companies located in industrialized countries may consume 60 to 65 percent of the world's silver, while thousands of medium- and small-sized companies, shops, and individuals, mostly engaged in silversmithing, jewelry manufacture, and the decorative arts, consume the remainder.

Metal traders of the world lists 104 silver bullion traders worldwide (Moreno, 1997, p. 586-589). In addition, 70 firms trade in doré, and 39 trade in semimanufactured forms of silver, such as sheet, strip, tubing, and wire. These are overlapping lists because many traders deal in more than one form of silver. The number of separate companies is also a little smaller than might be inferred from the above numbers because some of the larger traders have subsidiaries in several countries. Because the international trade in precious metals is so extensive, most silver traders can be assumed to be importers/exporters as well.

Although there is a sizable international market in silver-containing raw materials, the movement of a large part of the contained silver is not documented in trade statistics. The trade recorded for the 60 percent of the world's silver derived from base-metal deposits is that of the silver carriers (the base-metal concentrates, copper matte, lead bullion, and anode slimes), but the silver content is seldom stated.

By contrast, the movement of silver through the markets for silver bullion, semifabricated forms, and fabricated products, which also are international, is well documented in trade statistics. The cost of transportation in these markets is no impediment to international trade, being small in relation to the value of silver. Like gold bullion, silver bullion is being traded somewhere in the world at virtually every hour of the day. Silver is purchased by buyers from banks, bullion dealers, mining companies and refiners. Some of the silver bullion is shipped to the buyers, but some remains in the seller's storage vault after the transfer of ownership is documented. Some of the stored bullion may be shipped on a predetermined schedule or as the need arises, but other bars may remain in the vaults indefinitely, sometimes passing through several changes in ownership. Principal silver trading centers include Bombay, Hong Kong, London, New York, Shanghai, Singapore, Tokyo, and Zurich.

Futures trading in silver bullion is active around the world at commodity exchanges in such cities as Chicago, Hong Kong, London, New York, Tokyo, and Toronto. Over-the-counter and Exchange silver options are also traded in several of these cities.

Silver and the Environment

Most silver compounds, aside from those containing toxic anions, such as arsenate or cyanide, are essentially nontoxic. Compounds that dissociate in solution and provide significant concentrations of free silver ions can be toxic to bacteria and to freshwater aquatic organisms, but compounds and complexes in which the silver is tightly bound, such as silver sulfide and thiosulfate complexes, are innocuous. Nonetheless, a few other silver compounds must be handled with care. Strong silver nitrate solutions, for example, are caustic and highly irritating to eyes, mucosa, and skin (Cappel, 1997, p. 188).

The presence of silver in payable quantities does not appreciably change the environmental issues associated with the mining and processing of silver-bearing base-metal ores. Its presence in gold ores, however, can significantly increase the quantity of cyanide required to leach the ores. Procedures for dealing with environmental issues in mining and metallurgical extraction are well known and used, at least in the developed world. Most of the silver released into the environment comes from the manufacture and use of silver-containing products, of which the most important by far in this respect are photographic materials.

For economic reasons, virtually all the silver in spent thiosulfate photographic processing solutions is routinely recovered. The waste-water effluent from the recovery processes typically contains only from 0.1 to 20 mg/L of complexed silver (Cappel, 1997, p. 189). Microorganisms at waste-water treatment plants convert more than 90 percent of this to silver sulfide with some metallic silver; these insoluble products settle into the treatment plant sludge. Most of the remainder, which is discharged from secondary waste-water treatment plants, is tightly bound to sediment particulates, and does not subsequently enter the ground water (Thailand Environment Institute, undated).

Silver has no known physiological function in humans (Faust, 1992, p. 1). Most of the silver that enters the human body is breathed in or ingested in foods; a lesser amount enters through the skin. Some health problems have been experienced in the past by chemical workers who manufacture silver compounds, such as silver nitrate and silver oxide, and are exposed to dust that contains high levels of these products. Their symptoms included breathing problems, irritation of throat and lungs, and stomach pain (Agency for Toxic Substances and Disease Registry, 1999). Chronic exposure to silver and silver compounds can lead to the deposition of silver-protein complexes in body tissues. When deposited in the skin, these cause a permanent bluish-gray discoloration, which is termed "argyria." This is viewed as a cosmetic problem only; it appears to have no other adverse effect on health. In a related condition, argyrosis, silver is deposited in the conjunctiva and cornea of the eye; in some cases, it may impair night vision (Faust, 1992, p. 2; Cappel, 1997, p. 188). Effective workplace hygiene has made occupational argyria and argyrosis uncommon today. "Tests in animals show that silver compounds are likely to be life-threatening to humans only when large amounts (that is, grams) are swallowed, and that skin contact with silver compounds is very unlikely to be life-threatening" (Agency for Toxic Substances and Disease Registry, 1992).

In the United States, the level of silver in drinking water is not regulated, but the U.S. Environmental Protection Agency recommends that to avoid possible skin discoloration, silver should not exceed 0.01 mg/L. The Occupational Safety and Health Administration limits silver in workplace air to 0.01 milligram per cubic meter (mg/m^3) for an 8-hour workday, 40-hour workweek. The American Conference of Governmental Industrial Hygienists recommends that workplace air contain no more than 0.1 mg/m^3 metallic silver and 0.01 mg/m^3 soluble silver compounds (Agency for Toxic Substances and Disease Registry, 1999).

Supply, Demand, and Sustainability

World consumption (fabrication) of silver ordinarily outstrips what is sometimes termed "conventional" supply (mine production plus metal recycled from old scrap). The supply deficit is offset by sales of private and Governmental bullion stocks and by producer hedging. Individual countries may augment supply with net imports of silver. In the past decade, mine production has accounted for 60 to 67 percent of total annual world supply; secondary metal from old scrap, 19 to 23 percent; and bullion stocks and producer hedging, the remainder (Silver Institute, 2002b, p. 70).

The variable gap between conventional world supply and consumption for the past two decades is shown in figure 7. The record high silver prices of the late 1970s through the peak year of 1980 depressed consumption and increased the amount of scrap proffered to refiners, which led to surpluses during the 1980s. In the decade ending in 2001, the more usual supply deficits prevailed.

Exhibit H.

Memorandum from John H. Marburger, III, Director, Office of Science and Technology Policy and James L. Connaughton, Chairman, Council on Environmental Quality, to the Heads of Executive Departments and Agencies, Re: Principles for Nanotechnology Environmental, Health, and Safety Oversight (Nov. 8, 2007).

Executive Office of the President
Council on Environmental Quality

Executive Office of the President
Office of Science and Technology Policy

November 8, 2007

MEMORANDUM FOR THE HEADS OF EXECUTIVE DEPARTMENTS AND AGENCIES

FROM: JOHN H. MARBURGER, III
DIRECTOR, OFFICE OF SCIENCE AND TECHNOLOGY POLICY

JAMES L. CONNAUGHTON
CHAIRMAN, COUNCIL ON ENVIRONMENTAL QUALITY

SUBJECT: Principles for Nanotechnology Environmental, Health, and Safety Oversight

Nanotechnology is built on recent scientific advances that allow us to see, measure, and control matter at the scale of atoms and molecules. Such capabilities are enabling development of a variety of new products and processes with novel and potentially transformational characteristics. Advances in nanotechnology already are leading to applications in fields ranging from energy and environment to electronics and medicine. Realizing the benefits of nanotechnology will require not only research and development, but also appropriate oversight.

The Office of Science and Technology Policy (OSTP) and the Council on Environmental Quality (CEQ) led a multi-agency consensus-based process to develop a set of principles, shown below, to guide the development and implementation of policies for nanotechnology environmental, health and safety oversight at the agency level. This document is intended to summarize generally applicable principles relevant to such oversight for nanotechnology by the Federal government.

Federal agencies that have regulatory responsibilities, such as the U.S. Environmental Protection Agency, the U.S. Food and Drug Administration, the Occupational Safety and Health Administration, and the National Institute for Occupational Safety and Health, must implement sound policies to protect public health and the environment. In addition, agencies that perform nanotechnology research and development or that use nanotechnology in accomplishing their mission must provide appropriate oversight. These Federal agencies should follow the following principles as they develop policies for environmental, health, and safety oversight related to nanotechnology.

Principles for Nanotechnology Environmental, Health, and Safety Oversight

Purpose: Federal oversight approaches should be cognizant of the potential benefits of nanotechnology, including health, economic and environmental benefits, while recognizing uncertainties surrounding the evolving science and technology. The purpose of considering environmental, health and safety oversight approaches in the context of nanotechnology is to protect human health and the environment.

Current Understanding: The Federal government's current understanding is that existing statutory authorities are adequate to address oversight of nanotechnology and its applications. As with any developing area, as new information becomes available the Federal government will adapt or develop additional oversight approaches, as necessary, to address the area of nanotechnology.

Information Development: Adequate information should be developed with respect to the effects of nanomaterials on human health and the environment. To the extent practicable and respecting confidential information (e.g. Confidential Business Information (CBI)), this information should be developed in an open and transparent manner by stakeholders, including the Federal government and developers of nanomaterials.

Risk Assessment and Risk Management: The Federal government should use standard oversight approaches to assess risks and benefits, and manage risks, considering safety, health and environmental impacts, and exposure mitigation. As experience is gained, these approaches can be refined. The Federal government should strive to reach an appropriate level of consistency in risk assessment and management approaches across the government.

International: Recognizing the global efforts to develop nanotechnology, the Federal government should proactively promote international cooperation. The Federal government should encourage coordinated and collaborative health and environmental research and test data development across the international community. The Federal government should also promote access to information across the international community. These efforts will allow the Federal government to contribute to, and take advantage of, risk assessment and risk management approaches, as appropriate, across the international community.

Regulatory Path Forward: In light of the "Purpose" of oversight as described above, the Federal government should consider the following, to the extent permitted by law and where applicable, in establishing environmental, health, and safety regulations for nanotechnology:

- Regulation should focus where need exists and where scientific information supports action (e.g. targeted to specific groups and classes of materials instead of a "one-size-fits-all" approach);
- Decisions should be based on the best reasonably obtainable scientific, technical, economic, and other information;
- Where possible, regulatory approaches should enable rather than hinder innovation;

- Regulatory approaches should be performance based to the extent feasible and provide predictability and flexibility in the face of evolving science and technology;
- Benefits of regulation should justify their costs;
- Regulations should be developed in an open and transparent manner; and
- Regulations and guidance should consider established requirements and guidance such as the following:
  - Executive Order 12866 – Regulatory Planning and Review. Federal Register Vol. 58, No. 190, Monday, October 4, 1993, 51735-51744, available at http://www.whitehouse.gov/omb/inforeg/eo12866.pdf;
  - Information Quality Act (Sec. 515 of the Treasury and General Government Appropriations Act for FY 2001, Pub. L. No. 106-554); Information Quality Guidelines: OMB (2002) Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies (2002), 67 Fed. Reg. 8452 (Feb. 22, 2002) [hereinafter Information Quality Guidelines], available at http://www.whitehouse.gov/omb/fedreg/reproducible2.pdf;
  - National Technology Transfer and Advancement Act of 1995. Public Law 104-113, available at http://standards.gov/standards_gov/nttaa.cfm;
  - Office of Management and Budget (OMB) Circular A-119, Transmittal Memorandum, Federal Participation in the Development and Use of Voluntary Standards (02/10/1998), available at http://www.whitehouse.gov/omb/circulars/a119/a119.html;
  - OMB Final Information Quality Bulletin for Peer Review (December 16, 2004, available at http://www.whitehouse.gov/omb/memoranda/fy2005/m05-03.pdf;
  - OMB Bulletin No. 07-02 (M-07-07), Issuance of OMB's "Final Bulletin for Agency Good Guidance Practices" (January 18, 2007), available at http://www.whitehouse.gov/omb/memoranda/fy2007/m07-07.pdf; and
  - OMB/OSTP Memorandum: M-07-24, Updated Principles for Risk Analysis (September 19, 2007), available at http://www.whitehouse.gov/omb/memoranda/fy2007/m07-24.pdf

CHARLES L. FRANKLIN
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October 26, 2007

Mr. Marshall Swindell
Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard (South Bldg)
2777 Crystal Drive
Arlington, VA 22202

Re: Summary of October 16 Teleconference

Dear Marshall:

Thank you for your work in scheduling the presentation by Nanosilva, LLC ("Nanosilva") to the OPP Nanotechnology Committee (the "Committee") for November 7, 2007. We look forward to the opportunity to present Nanosilva™ Antimicrobial (the "Product") to the Committee and to move forward with the serious work of registering OPP's first true pesticide product that utilizes nanotechnology. In that vein, below is a summary of key issues discussed during our teleconference on Tuesday, October 16, 2007, and our understanding of the next steps in the review and consultation process.

I. Minutes of October 16 Teleconference

On October 16, 2007, Wayne Krause of NanoSilva and Charles Franklin of Akin Gump Strauss Hauer & Feld LLP ("Akin Gump") held a teleconference with Marshall Swindell of the Antimicrobials Division of EPA's Office of Pesticide Programs ("OPP"). During the teleconference, Mr. Swindell provided a preliminary review of the administrative portion of NanoSilva's draft application package. He also provided recommendations as to what information would be needed for the Presentation to the OPP Nanotechnology Committee.

A. Qualification of NanoSilva as a "Nanotech" Pesticide

Mr. Swindell noted that a number of companies have approached OPP regarding so-called "nanonotech" products but, to date, none has met EPA's stringent nanotech standard or been formally submitted for registration review. Mr. Swindell indicated that

given the size, unique function, and manufacturing process for Nanosilva™, the product likely meets the Agency's definition. The NanoSilva presentation should address these criteria for the benefit of the Committee.

B. Status of OPP's Nanotechnology Policy

Mr. Swindell stated that EPA is still developing its policy regarding when to regulate nanoscale versions of currently-registered active ingredients as new uses versus as new chemicals. Mr. Franklin noted that during an earlier conversation, Dennis Edwards had indicated that EPA would make these determinations on a case-by-case basis, and that NanoSilva would have the opportunity to make a case for "new use" treatment, given the Product's low toxicity and low exposure risk, and the presence of so many other silver-based registered antimicrobial products. Mr. Swindell recommended that NanoSilva emphasize these issues in the presentation to the Committee.

C. Data Requirements

Mr. Swindell reviewed the proposed Data Matrix and noted that NanoSilva should be prepared during the Presentation to discuss any "Tier 1" data requirements not currently listed in the Application. In particular, he identified: 1) a 90-day dermal study; 2) one or more mutagenicity studies; 3) a teratogenicity study; and 4) various other animal studies assessing toxicity to fish, birds, and invertebrates. In each case, the Company would need to address the requirement either by justifying a waiver, identifying existing data appropriate to support OPP's review, or by submitting the data directly. Mr. Swindell also recommended that the Company discuss any data addressing the Product's low leachability and exposure risk. Mr. Swindell confirmed that because the Company is seeking a treated article registration only, microbiological data will not be required as part of the registration package. NanoSilva should, however, maintain efficacy data as part of its supporting files and will need to submit such data if the Company later seeks to make "public health" claims.

D. Mode of Action for the Active Ingredient

Mr. Swindell requested further detail regarding the mode of action that gives NanoSilva™ Antimicrobial its pesticidal effect. Mr. Krause described the process and the historic data that address the mode of action. Mr. Swindell recommended that NanoSilva discuss the mode of action in the presentation.

E. Labeled Uses on the Technical Bulletin

Mr. Swindell reviewed the Technical Bulletin and noted several listed uses that may need to be clarified to avoid triggering the Product's characterization as a "food use." Examples included references to "conveyer belt," "countertops," and "paper." NanoSilva confirmed that it did not intend such uses to extend to food-uses. Mr. Swindell also noted that the references to "Drinking Water Contact Uses" would typically trigger a "food-use" finding and the requirement for a supporting dietary safety determination and establishment of a tolerance or tolerance exemption. In light of the product's nonleachability and concomitant minimal exposure risk, however, the Committee may be willing to waive such food-use data requirements.

II. Next Steps

Using your feedback and direction from the October 16, 2007 teleconference, NanoSilva is now actively involved in preparing for its presentation to the Committee on November 7, 2007. As requested, NanoSilva will provide a copy of its presentation materials in early November to assist the Committee in its own preparations for the meeting. In the meantime, if you have any questions regarding NanoSilva's product, its advance application, or its preparations for the November 7 meeting, please contact me at your convenience. Thank you.

Regards,

Charles L. Franklin

NANOSILVA, LLC

Document Processing Desk (REGFEE)
Attn: Marshall Swindell
Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard (South Bldg)
2777 Crystal Drive
Arlington, VA 22202

September 25, 2007

Subject: New Product - New Use (A44-A52 PRIA), EPA Reg. No._____

Dear Mr. Swindell:

As you requested, enclosed is an advance draft of key portions of the Nanosilva, LLC ("Nanosilva") registration application for Nanosilva™ Antimicrobial, a silver silica colloid with potential uses in a variety of treated article applications.[1] We appreciate your offer to review these key documents, and to work with your colleagues on OPP's Nanotechnology Committee to explain our product's unique characteristics. With a considered understanding of our product and its reduced-risk profile, we are confident that the EPA will find it to be a model for future pesticide products of nanotechnology origin.

This advance review package includes the following documents:

- Transmittal document.
- EPA Application Form 8570-1, one (1) copy.
- Product label, one (1) copy.
- Confidential Statements of Formula EPA 8570-4, one (1) copy.
- Data Matrix Table EPA Form 8570-35, one (1) copy.[2]
- Nanosilva™ Antimicrobial Technical Bulletin, one (1) copy.
- Nanosilva ™ Antimicrobial Material Safety Data Sheet, one (1) copy.

As outlined in this package, and supported by the data to be submitted with the full formal registration application, Nanosilva™ Antimicrobial combines the three key

[1] As part of the full application, Nanosilva will submit the additional supporting data addressing chemistry, product safety and toxicology studies, along with other required administrative forms and waiver requests, including a small business waiver under PRIA.

[2] No efficacy studies are submitted with this application because this product is intended for use in treated articles. We also intend to register this product with the Food and Drug Administration as a Food Contact Substance.

qualities that the EPA has emphasized under its pollution prevention program: low toxicity, low concentration, and low exposure risk.

This technology was developed in 2002 by Dr. Seong Oh, Vice Dean and Ph.D., Materials Science, Hanyang University, Seoul, Korea, who is also the originator of this compound. The unique aspect of this technology is retention of the silver particle as the primary and most important characteristic. The silver molecule does not leach out of the concentrate colloidal form, the polymeric intermediate form or the treated material. This characteristic sets Nanosilva™ Antimicrobial apart from "silver-ion" or other "nano-silver" marketed antimicrobial material.

The technology presented in this application for EPA registration of Nanosilva™ Antimicrobial represents a covalently bonded silver-silica colloid. This is a uniquely stable bonded use of nanosilver particles coupled to a larger nanosilica particle. The novel preparation and nature of stable chemical bonding maintains a non-leachable form of silver attached to a silica particle. When silver in this form is introduced into a polymer, the covalently bonded silver contained within the plastic polymer is able to exert antimicrobial characteristics by conversion of molecular oxygen to a short-lived free radical form of Oxygen molecule at or near the polymer/air interface. This is accomplished by an energy conversion at the molecular level which is unique to this product.

Another strategic characteristic of this technology is the minimal toxicity displayed by the extremely low concentration levels of silver required to demonstrate the intended effect in the finished treated article. The silver content present in the silver-silica colloid is only 1.00% by weight. The silver concentration is further reduced by 90-95% during the formulation of the polymeric intermediate (master batch). Once formulated, the polymeric intermediate is then integrated into the finished treated article at 5-10% concentration by weight, resulting in silver concentration levels of 8-16 parts per million in the final product, bound and chemically stable with no detectable leaching.

We are aware that the possible release of silver nano-particles into the environment has been a source of recent concern to the EPA, particularly with 'silver-ion' technologies. Such concerns are inapplicable to the Nanosilva™ technology. Unlike products that rely on the release of silver ions for their effect, *see, e.g., http://www.epa.gov/oppad001/ion_gen_equip.htm,* the silver molecules in Nanosilva™ Antimicrobial do not leave the article to provide benefits and, indeed, have proven to be non-leaching to the method detection limit at parts per billion (ppb) levels during testing. (OPPTS 830.6317 (Storage Stability) and 830.6320 (Corrosion Characteristics) were initiated on a start date of June 13, 2007).

In closing, the uniquely stable bond between the nanosilver and the silica particle minimizes the silver's leachability and any related risks of migration into the environment, both in the colloid form and in the final article. Indeed, the stable,

nontoxic, and nonleaching characteristics of the silver-silica compound contained in Nanosilva™ Antimicrobial should mitigate the need for more extensive environmental fate studies.

Thank you again for your efforts to promote a timely and tailored review of this product. We welcome your feedback on this advance submission and look forward to working with you and your staff to register this product. If you have any questions, please contact me at your convenience. Thank you.

Regards,

Wayne Krause
Vice President Operations
Phone: (352)-615-4906
Fax: (352)-368-1796
wkrause@clairson.com

Enclosures

Exhibit A.

NanoSilva, LLC, Advance Draft Registration Application for NanoSilva™ Antimicrobial, provided by Wayne Krauss, VP Operations, NanoSilva, LLC to Marshall Swindell, Office of Pesticide Programs, EPA (Sept. 25, 2007).

NANOSILVA, LLC

Document Processing Desk (REGFEE)
Attn: Marshall Swindell
Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard (South Bldg)
2777 Crystal Drive
Arlington, VA 22202

September 25, 2007

Subject: New Product - New Use (A44-A52 PRIA), EPA Reg. No._____

Dear Mr. Swindell:

As you requested, enclosed is an advance draft of key portions of the Nanosilva, LLC ("Nanosilva") registration application for Nanosilva™ Antimicrobial, a silver silica colloid with potential uses in a variety of treated article applications.[1] We appreciate your offer to review these key documents, and to work with your colleagues on OPP's Nanotechnology Committee to explain our product's unique characteristics. With a considered understanding of our product and its reduced-risk profile, we are confident that the EPA will find it to be a model for future pesticide products of nanotechnology origin.

This advance review package includes the following documents:

- Transmittal document.
- EPA Application Form 8570-1, one (1) copy.
- Product label, one (1) copy.
- Confidential Statements of Formula EPA 8570-4, one (1) copy.
- Data Matrix Table EPA Form 8570-35, one (1) copy.[2]
- Nanosilva™ Antimicrobial Technical Bulletin, one (1) copy.
- Nanosilva ™ Antimicrobial Material Safety Data Sheet, one (1) copy.

As outlined in this package, and supported by the data to be submitted with the full formal registration application, Nanosilva™ Antimicrobial combines the three key

[1] As part of the full application, Nanosilva will submit the additional supporting data addressing chemistry, product safety and toxicology studies, along with other required administrative forms and waiver requests, including a small business waiver under PRIA.

[2] No efficacy studies are submitted with this application because this product is intended for use in treated articles. We also intend to register this product with the Food and Drug Administration as a Food Contact Substance.

qualities that the EPA has emphasized under its pollution prevention program: low toxicity, low concentration, and low exposure risk.

This technology was developed in 2002 by Dr. Seong Oh, Vice Dean and Ph.D., Materials Science, Hanyang University, Seoul, Korea, who is also the originator of this compound. The unique aspect of this technology is retention of the silver particle as the primary and most important characteristic. The silver molecule does not leach out of the concentrate colloidal form, the polymeric intermediate form or the treated material. This characteristic sets Nanosilva™ Antimicrobial apart from "silver-ion" or other "nano-silver" marketed antimicrobial material.

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Thank you again for your efforts to promote a timely and tailored review of this product. We welcome your feedback on this advance submission and look forward to working with you and your staff to register this product. If you have any questions, please contact me at your convenience. Thank you.

Regards,

Wayne Krause
Vice President Operations
Phone: (352)-615-4906
Fax: (352)-368-1796
wkrause@clairson.com

Enclosures

NANOSILVA, LLC
2811 NE 14TH STREET
OCALA, FL 34470

Antimicrobial Additive

Technical Bulletin

NANOSILVA™ EPA Reg. xxxxxxxx

NANOSILVA™ is a covalently bound, silver-silica based antimicrobial additive engineered through proprietary developments in nanotechnology and is designed for integrated use in the manufacture of polymer, plastic and textile products.

NANOSILVA™ suppresses the growth of bacteria, algae, fungus, mold and mildew, which can cause unpleasant odors, discoloration, staining and deterioration of those manufactured products.

**Finished products containing NANOSILVA™ antimicrobials may not make public health claims relating to antimicrobial activity without EPA pesticide registration. When used in treated articles, this product does not protect users of any such treated article or others against food borne or disease causing bacteria, viruses or other
disease causing organisms.**

NANOSILVA™ Antimicrobials may be used in material that may be incorporated into the finished product at 5.00±10% of NANOSILVA™ Antimicrobial by weight. Contact NanoSilva to determine the appropriate amount of NANOSILVA™ Antimicrobial for individual finished products.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

NON-FOOD CONTACT USES: APPLICATIONS AND TYPES OF MATERIALS

Plastics including Films, Sheets, Slabs and Molded Plastic Parts – appliances and equipment; automobile parts; brush bristles; brush handles; building materials (such as hardware, handles); conveyor belts; countertops; floor coverings; flooring; footwear (including boots, sports, equipment and tools); gaskets; general purpose containers; indoor and outdoor furniture; insulation for wire and cable; insulators; kitchen and bathroom hardware; liners; mats; mops; non-woven fabrics; plastic film; plumbing supplies and fixtures (including toilet bowl seats); protective covers; shower curtains; siding for housing; sinks; sponges; tape; tiles; tubing; vacuum cleaner bags; waste containers.

Fibers – apparel (such as umbrellas, outerwear, sportswear, sleepwear, socks and hosiery, caps, undergarments, inner liners for jackets, trim for outerwear and garments, uniforms, outerwear, gloves, aprons, coats and shoes); conveyor belts; industrial and other household items (such as artificial leather, filters, book covers, mops, cloth for sails, ropes, tents and other outdoor equipment, tarps, awnings, bags, brush bristles, commercial and industrial wipes and fabrics, sponges, wiping cloths); interior furnishings (such as mattress cover pads and filling, pillow covers, sheets, blankets, fiberfill for quilts and pillows, curtains, draperies, carpet and carpet underlay, rugs, upholstery, towels, wall covering fabrics, cushion pads, sleeping bags); packaging; transportation items (such as automotive and truck upholstery, carpeting, rear decks, trunk liners, convertible tops, interior liners).

Coatings, Films and Laminates – appliances; automotive and vehicle parts; barrier fabrics; building materials and components (such as walls, wallboard, floors, concrete, siding, roofing, shingles, hardware, floors, ceilings, commercial and industrial applications); collection and storage equipment (such as conveyor belts, piping systems, silos, tanks and process vessels); countertops; furniture; general purpose containers; glazing for cement tile; glazing for vitreous china used in plumbing fixtures (such as toilets, sinks and countertops); industrial equipment; natural and synthetic fibers and fabrics; packaging; paper products (such as wipes, tissues, wall coverings, towels, book covers); sinks.

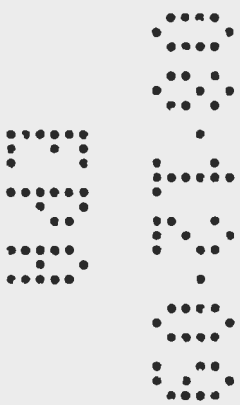
Adhesive and Sealants – appliances; automotive and vehicle parts; bathrooms; cardboard; ceramic tile; construction materials; glazing for windows; grout and joint compound; paper; pipe sealant and insulating materials; plastic; plumbing adhesives; rubber; sealants for pipes; showers; wood; wood and plastic composites.

Miscellaneous Applications – cat litter; drainage and sewage pipe; flooring; plaster; sinks; stucco; tile; toilets.

Heating, Ventilation and Air Conditioning (HVAC) – HVAC equipment and related materials including air handlers, plenums, coils, fins, insulation, rigid or flexible ducts, drain pans, duct support mechanisms, diffusers, filters, heat exchangers, air purifiers, supplemental articles used in the assembly of HVAC systems such as gaskets, fixtures, sealants, adhesives and HVAC reinforcing, as well as parts and components thereof. These articles could be constructed of fibrous materials (textiles), non-woven, plastic or coated metal material. For use only during manufacture of the article.

DRINKING WATER CONTACT USES: NanoSilva™ may be used for the human drinking water contact uses listed below. The additive may be incorporated into food bowls and water bowls, dishes and other containers used by domestic animals. Do not use for any food or drinking water applications involving non-domestic animals.

Plastics, Fibers, Coatings, Films and Laminates, Adhesives and Sealants – including ice making equipment (water pans, piping, tubing, guards, ice storage bins, trays, ice scoops, buckets, valves and gaskets); drinking water contact materials (water bottles, cups, gaskets, plumbing fixtures, storage tanks and vessels, water piping, tubing, valves, spigots, coolers, water dispensing components, housing units and water filter components).



MATERIAL SAFETY DATA SHEET

SECTION I. CHEMICAL PRODUCT and COMPANY IDENTIFICATION

Manufactured by: NanoSilva, LLC
Product Code: NSPW L30SS
Product Name: **Nanosilva Silver-Silica Colloid – 1.0% Solution in Water / Ethylene Glycol.**
Synonyms: Silver-Silica Colloid
Chemical Family: Inorganic Silver and Silica – Elemental

SECTION II. COMPOSITION and INFORMATION on INGREDIENTS

Chemical Name: Silver-Silica Colloid
Formula: $Ag_{0.001\text{-}0.01\mu}SiO_2$
Molecular weight: 60.198 – 61.168
Components: Silver-Silica (covalently bound), Ethylene Glycol and Water

SECTION III. HAZARDS IDENTIFICATION

Acute Toxicity: (oral)	Acute Oral LD50: 100% survival at 5000 mg/Kg (Mouse)
Acute Toxicity: (dermal)	Acute Dermal LD50: 100% survival at 5,000 mg/Kg (rat)
Chronic Toxicity	None
Inhalation (acute)	LC50 greater than 2.07 mg/L
Ingestion:	Acute Oral LD50 greater than 5,000 mg/Kg (rat)
Skin:	Not a Contact Sensitizer, Buehler Method
Dermal Irritation	(PDII): 0.3 average score, slightly irritating clears in 72 hrs.
Eyes (primary irritation)	MMTS: 16.7 (Average Irritation Score), Mildly irritating
Unusual chromic toxicity:	None
Potential Acute Health Effects	Carcinogenic Effects: not determined
Potential Acute Health Effects	Mutagenic Effects – not determined
Teratogenic Effects	Not available.
Developmental Toxicity	Not available.

SECTION IV. FIRST AID MEASURES

Eye Contact	Flush eyes with low pressure water for at least 15 minutes.
Skin Contact	Wash skin with soap and water, remove contaminated clothing.
Inhalation	not applicable
Ingestion	Rinse mouth and throat thoroughly with tap water, seek medical attention.

SECTION V. FIRE AND EXPLOSION HAZARD DATA

Flammability of Product	Non-flammable
Auto-Ignition Temperature	Not applicable
Flash Point:	Not applicable
Unusual Fire and Explosion Hazards:	None
Fire Extinguishing Agents recommended:	Non-flammable

SECTION VI. ACCIDENTAL SPILL OR LEAK PROCEDURES

Personal Precautions:	Avoid contact. Containment and clean up should be performed by personnel wearing suitable hand and skin protection. This includes latex or vinyl gloves, ANSI approved safety goggles, NIOSH/MSHA approved high efficiency particle Respirator.
Small Spill	Mop up, or absorb with an inert dry material and place in an appropriate container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local regional authority requirements.
Large Spills	Absorb with an inert material and put the spilled absorbed material in an appropriate waste disposal. Finish cleaning by spreading water on the spill surface and allow to discharge through the sanitation system.

SECTION VII. HANDLING and STORAGE

Precautions	Do not ingest, wear latex or vinyl gloves, avoid skin exposure.
Storage	Keep container tightly closed, keep container in a cool area.

SECTION VIII. EXPOSURE CONTROLS / PERSONAL PROTECTION

Eyes:	Flush immediately with water for 15 minutes holding eyelids open.
Skin:	Remove contaminated clothing, wash skin.
Ingestion:	Administer 2-4 cups of milk or water. Seek medical attention.
Respiratory Protection:	Dilute solution no respiratory exposure.
Eyes and Face:	Use glasses, goggles or face shields to prevent eye exposure.
Ventilation:	Use adequate ventilation no harmful vapor associated with solution.

SECTION IX. PHYSICAL DATA

Appearance and Odor:	Yellow opaque liquid with no odor.
Boiling Point:	101 C
Density:	0.99 To 1.01 mg/ ml
pH of 1.0% Solution:	2.5 – 3.5
Volatility:	Non-volatile
Solubility in water:	Insoluble in water, dispersed as particles
Viscosity:	3,800 cps. (Brookfield, Spindel # 5, 100 RPM)

SECTION X. STABILITY and REACTIVITY DATA

Stability:	The product is stable
Conditions to avoid:	Halogen salts, Chlorides will precipitate as AgCl.
Hazardous decomposition products:	None
Incompatibility:	None

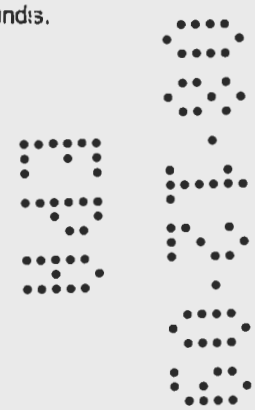
SECTION XI. TOXICOLOGICAL INFORMATION

Toxicity to Animals	Acute Oral LD50: 100% survival at 3000 mg/Kg (Mouse) Acute Dermal LD50: 100% survival at 5,000 mg/Kg (rat) Inhalation LC50: greater than 2.05 mg/L
Primary Irritant effect	Skin: Not a Contact Sensitizer, Buehler Method Eye: minimally-irritating, MMTS: 8.0 average score
Sensitization	No sensitizing effects known.
Carcinogenic Effects:	Not determined
Mutagenic Effects:	Not determined

Legal responsibility is assumed only for the fact that all studies reported here and all opinions are those of qualified experts. Buyer assumes all risks and liabilities. He accepts and uses this material on these conditions. He must have a copy of this MSDS where this material is handled.

SECTION XII. ECOLOGICAL INFORMATION

Ecotoxicity:	Not determined
Products of Degradation:	Non-Toxic degradation to basic elemental composition.
Products	Non-toxic
Remarks on Products Biodegradation.	Degradation will follow the path of elemental compounds.



SECTION XIII. DISPOSAL CONSIDERATIONS

Waste disposal must be in accordance with federal, state and local environmental control regulations.

SECTION XIV. TRANSPORT INFORMATION

DOT Classification: Not a DOT controlled material.
\

SECTION XV. REGULATION

Hazardous Symbols: None required.

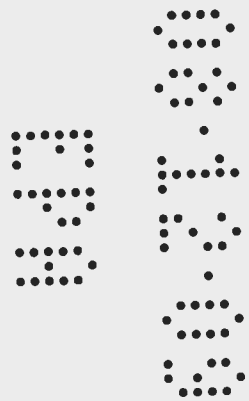
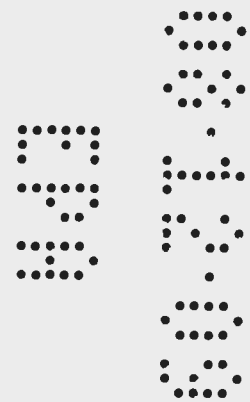


Exhibit B.

NanoSilva™ Antimicrobials, Presentation to the EPA Office of Pesticide Programs Nanotechnology Committee (Nov. 8, 2007).



CHARLES L. FRANKLIN
1.202.887.4378/fax: 1.202.887.4288
clfranklin@akingump.com

March 5, 2008

HAND DELIVERY

Mr. Marshall Swindell
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard (South Bldg)
2777 Crystal Drive
Arlington, VA 22202

Re: Pre-Application Review Package for NanoSilva Antimicrobial.

Dear Marshall:

Enclosed is the draft registration package for NanoSilva™ Antimicrobial, authorized for distribution to the OPP Nanotechnology Committee in preparation for our pre-registration meeting to discus a path forward on the Product's registration process. This package contains the materials required to support the Company's application for new-use registration of this product, as established during pre-registration meetings in January and February 2007. Specifically, this package includes:

- A short one-page summary of key facts supporting the proposed application;
- The draft application form and supporting administrative attachments;
- Data addressing the Product's identity (Vol. 1);
- Data addressing the Product's physical and chemical properties (Vol. 2); and
- Data addressing the acute toxicity profile for the Product (Volume 3)

The Company is also preparing, for hand delivery by this time next week, supplementary information on the product's leachability and responses to specific questions submitted by Mr. Demson Fuller on February 29, 2008. Consistent with your February 28 counterproposal, we would like to schedule a meeting with you and Committee staff between March 17 -19 or March 24-26, Please let me know which dates and times would work for the Agency. Thank you.

Regards,

Charles L. Franklin

Enclosures

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Summary of Key Application Facts

Product Identity and Characteristics

- Active ingredient is elemental silver (derived from silver nitrate and manufactured into nanoclusters through proprietary closed-loop process).
- Silver nanoclusters are covalently bound to silica particles using sulfur as a non-reactive molecular tether.
- Covalently- bound silver clusters demonstrates high stability in silver-silica colloid. Once incorporated into polymer substrate, there is no detectable leaching.
- Silver silica colloid contains silver at 8,000 ppm. Intermediate master batch contains 160 ppm. The final treated article contains only 8-20 ppm.

Incorporated Into the Treated Article

- Nanosilver clusters within the polymer matrix react with oxygen at the surface of the article to create very short-lived oxygen radicals that interact with microorganism cells.

Proposed Uses

- Registered as colloidal solution (at EPA's suggestion) but blended into solid polymer intermediate "master batch" for sale and distribution to processors.
- Labeled for use in treated articles to suppress growth of bacteria, algae, fungus, mold and mildew in polymers, plastics, and textiles. No public health claims.
- Proposed for use sites already approved for other silver-based treated article antimicrobials.

Risk Profile

- Acute toxicology tests indicate mild ocular, low dermal, and absence of oral, inhalation, or contact sensitization toxicity.
- Data on other silver-based treated article products confirm that silver poses minimal subchronic or chronic risks even when used at much higher concentrations and levels of bioavailability.
- OPPTS registration testing shows solution is insoluble and would quickly settle as sand with no leaching.
- Immobility of bound-silver in this product mitigates risk from sub-chronic or chronic exposure to nanoscale particles.

[redacted]

NANOSILVA ™

ANTIMICROBIALS

A New Standard In Performance and Protection

Father of Technology

Dr. Seong Oh

PhD in Chemical Engineering from University of Florida

Current Dean of Admissions Hanyang University Seoul, Korea

Holds 31 combined U.S. and Korean patents

Research Fields:

- Preparation of metal/metal oxide nanoparticles using surfactants
- Preparation of functionalized nanoparticles
- Preparation of nanostructured materials using surfactant aggregates as nanoreactors
- Synthesis of organic/inorganic nanocomposites
- Synthesis of functionalized surfactants

Published over 100 research papers in field of study

20 years experience in nanotechnology research and development

Birth Place of Technology
Hanyang University

- College of Engineering
- Private University
- Located in Seoul, Korea
- Founded in 1934
- Facility and Student Body: 46,000

[redacted]

History of Technology
(Research and Development)

- Research and Development began in 1997
- First Korean patent issued in 2000
- First U.S. patent issued in 2002
- Technology first commercialized in 2002 (NanoBio Ltd.)
- Technology Exclusively Licensed to NanoSilva LLC in 2005
- Technology Trade Marked as Nanosilva™ in 2006
- Current fields of use (Asian-Pacific Region): Cutting Fluids, Paints, Consumer Polymeric based products.

History of Technology
(Regulatory Consultations)

- Consultations with EPA Office of Pesticide Programs
  - Initial Consultation (Jan. 2007)
  - Follow up Meeting (Feb. 2007)
  - Review of Administrative Package (Oct. 2007)
  - Presentation to OPP Nanotechnology Committee (Nov. 2007)

- Consultations with Food and Drug Administration
  - Initial Consultation (Jan. 2007)
  - Presentation to FDA Nanotechnology Work Group (Jan. 2007)
  - Follow up Meeting (Mar. 2007)
  - Up date conference (Sept. 2007)

- Presentation to ASTM Committee on Antimicrobials (Oct. 2007)

Nanosilva™ Introduction

- Revolutionary silver-silica based treated article additive.
- Engineered using proprietary developments in nanotechnology.
- Qualifies as nanotechnology product under white paper.
- Protects product while exhibiting unique structural stability, low leachability, and low toxicity.
- Alternative to conventional leaching or migratory type synthetic organic agents and ionic silver based technologies.
- Registered as colloidal solution and distributed in custom-formulated polymeric intermediates (Master batches).

[redacted]

Nanosilva™ Characteristics

- Active Ingredient: Elemental Bound Silver
- Antimicrobial effect is oxygen activated
- Particle size: 30-50 nanometers
- Concentration Level:
  - **A.)** Colloidal form: 8,000 ppm
  - **B.)** Intermediate form (Master batch): 160 ppm
  - **C.)** Final treated article: 8 ppm – 20 ppm

Nanosilva™ Proposed Use – Treated Article

- Reduces odor development
- Suppresses Bio-film formation
- Eliminates discoloration and staining
- Protects against premature degradation
- Supplements normal hygienic practices

Core Technology – Bound Silver
(Silica-Sulfur-Silver Complex)

CHARACTERISTICS

1) Supporting structure is a synthesized silica nanoparticle.
2) Sulfur acts as a tether to chemically bind silver nano-clusters to nano-silica particle.
3) Silver nano-clusters consist of approximately ten (10) atoms of silver which are metallically bound.
4) By design, silver nano-clusters are covalently bound to sulfur and sulfur is covalently bound to nano-silica particle.
5) The number (coverage) and size of immobilized particles can be controlled by adjusting reaction rate.

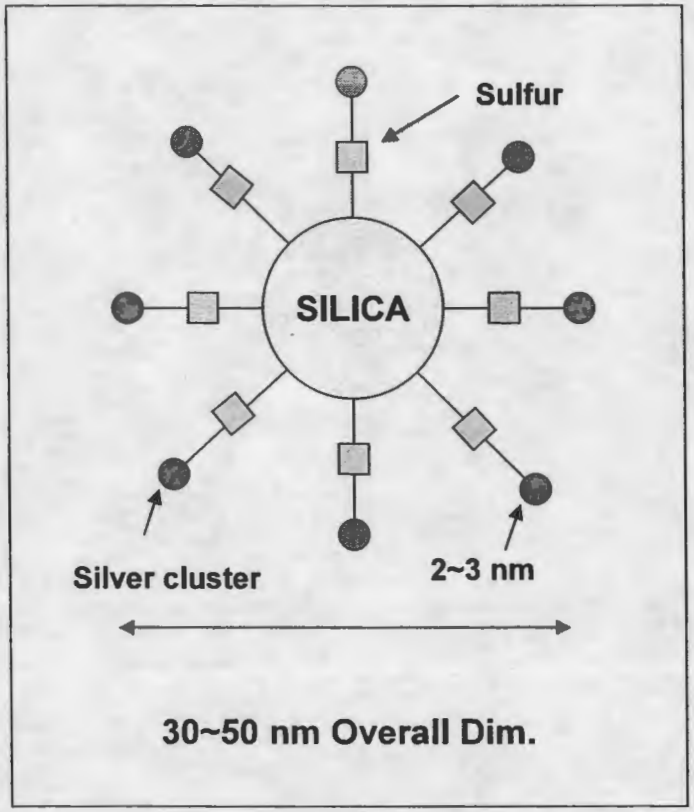
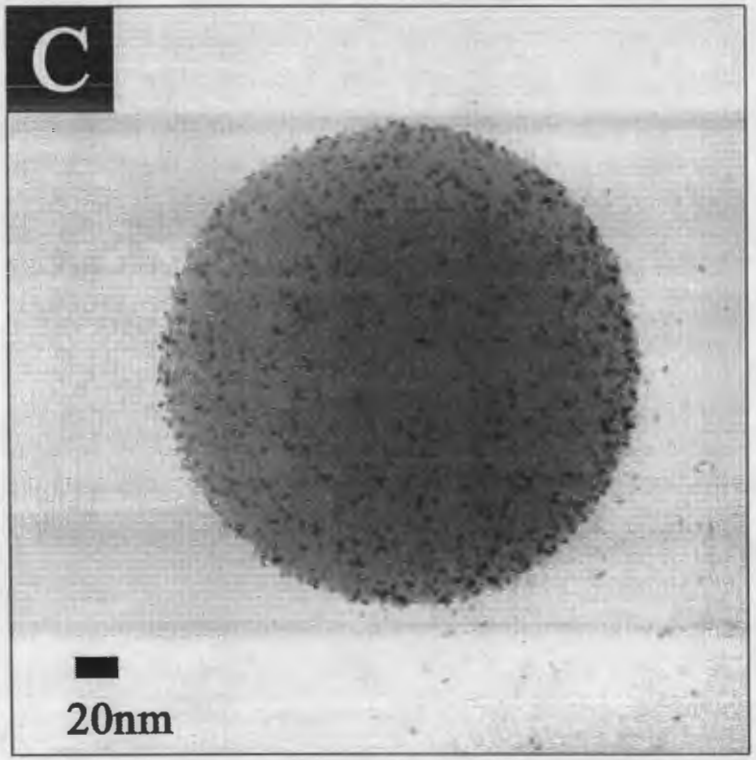
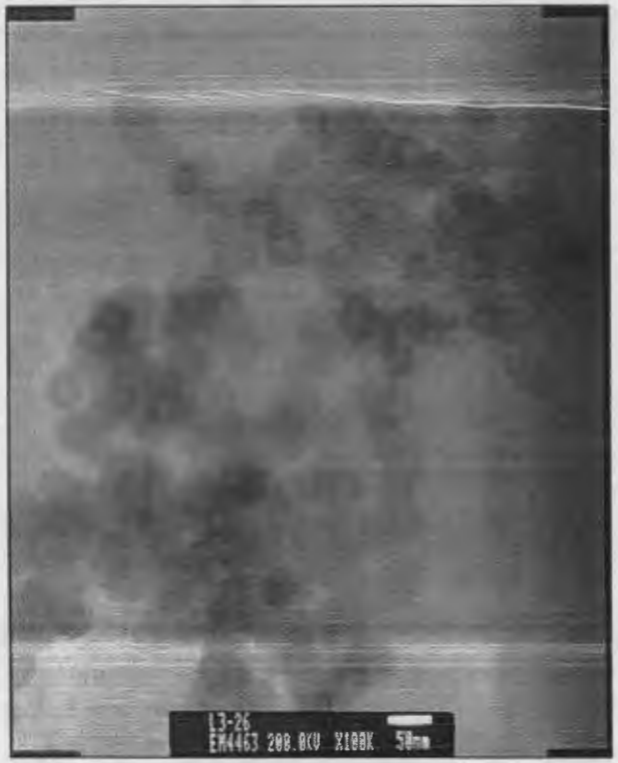


Diagram of complex particles

Core Technology-Bound Silver



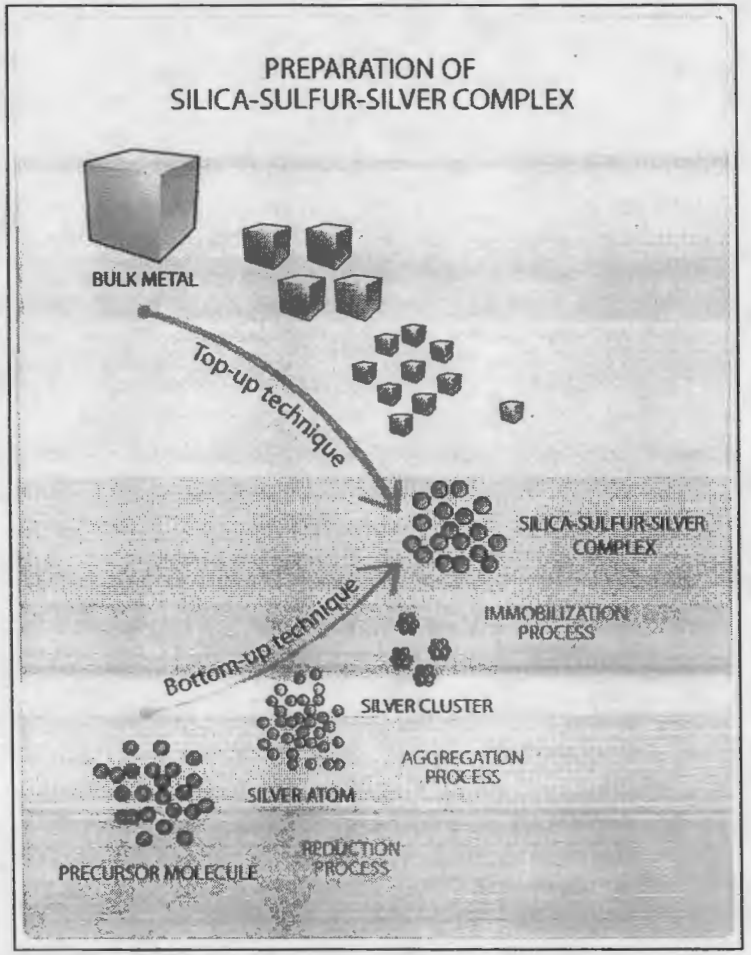


TEM images of Nanosilva™ complex particles

[redacted]

Preparation Of Silica-Sulfur-Silver Complex

- Wet Chemical Synthesis Technique
  - Reduction of silver precursor molecules to silver atoms
  - Nucleation and growth of silver nano-clusters
  - Immobilization of silver nano-clusters on the surface of modified silica nanoparticles



Preparation of Intermediate

A

High speed / homogeneous mixing

Blend solution with granulated Form of specified polymer to Form slurry.

B

High speed / homogeneous mixing

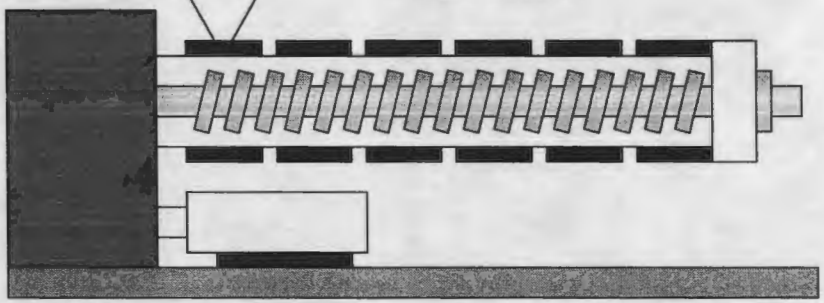
Blend slurry with palletized Form of specified polymer

C

Extrusion

D

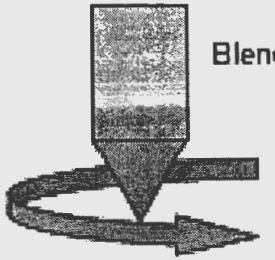
Polymeric Intermediate (Master Batch)



Solvents and by products evaporate during extrusion process

Incorporation into Treated Articles

A



Blender

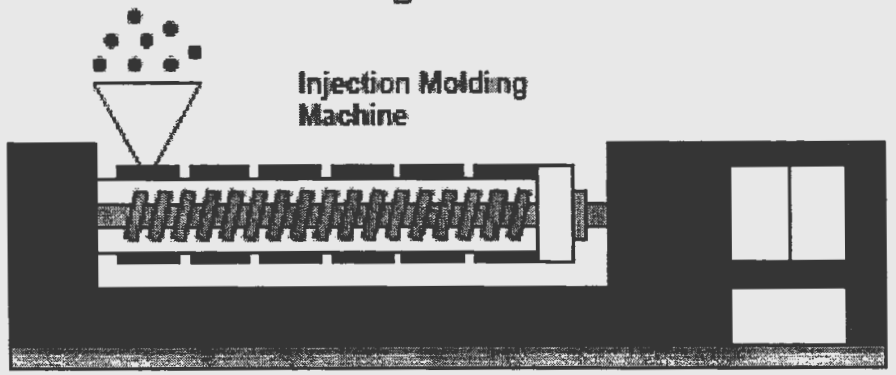
Master Batch is blended
With base polymer at a 5%
Let-down

Colorant may be added
During this stage

Optional Gravimetric blender maybe utilized
Directly attached to machine

B

Injection Molding
Machine



C

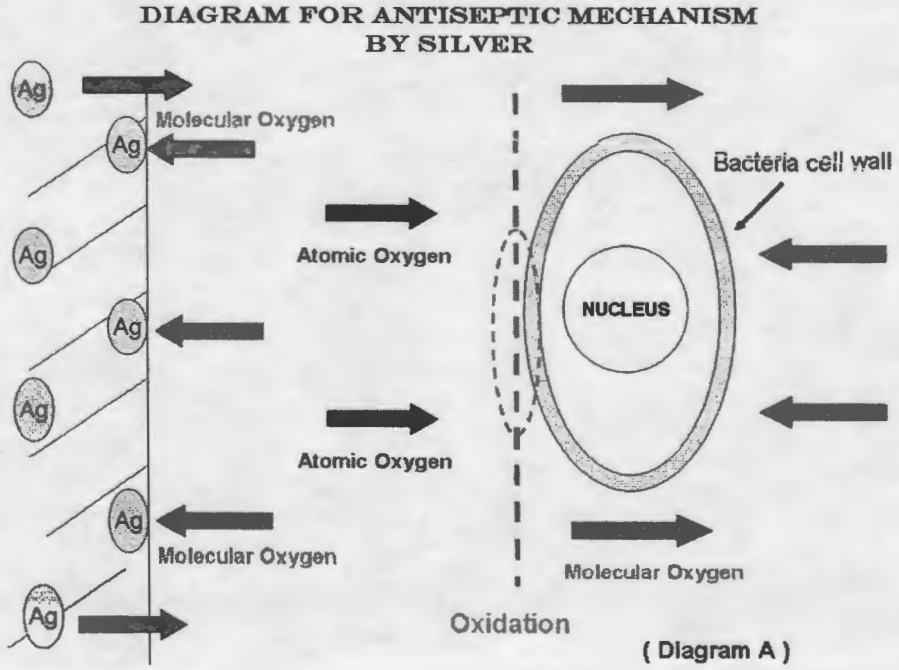
FINISHED TREATED
ARTICLE

Optional Molding Techniques
Blow Molding
Rotational Molding
Profile Extrusion
Vacuum Forming

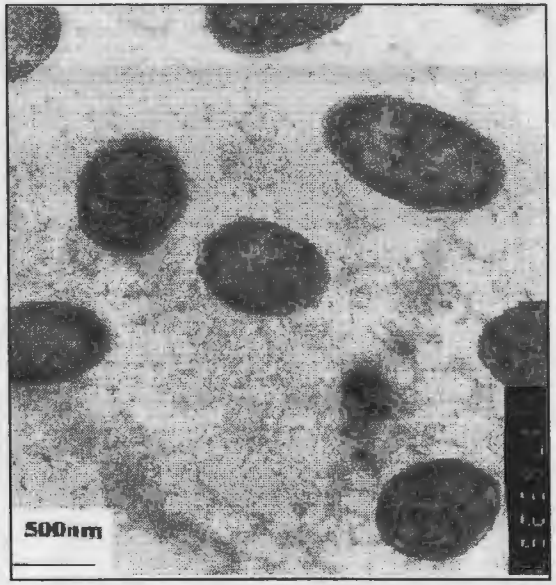
[redacted]

Mode Of Action for Treated Article

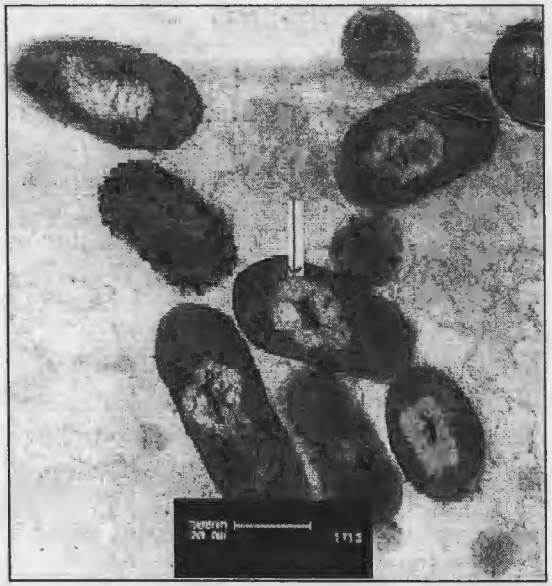
- Description of Antimicrobial Mechanism By Bound Silver
  - Molecular oxygen dissociates upon contact with silver.
  - Atomic oxygen absorbs onto the surface of the silver.
  - Atomic oxygen contacts microorganism and removes hydrogen (H) from sulfhydryl groups on the surface.
  - Sulfur forms an R-S-S-R bond which effectively blocks respiration of microorganism



Mode of Action for Treated Article



Normal Cells



Cells Exposed to Treated Article

Cell Wall Destruction

[redacted]

Nanosilva™ Antimicrobial

- Product of nanotechnology
- Structural stability
- Low concentration levels
- Low toxicity profile
- Non-leaching/non-migrating

Nanosilva™ Antimicrobial

Laboratory Analysis and Findings Summary

Dr. Peter J. Kmieck
Director, Kappa Laboratories, Inc.

Kappa Laboratories, Inc.

2577 NW 74 Avenue
Miami, Florida 33122

Mt. Sinai Medical Center
Biomedical Research Building
4300 Alton Road
Miami Beach, Florida 33140

- Food and Drug Testing
  - Kappa Laboratories is a full-service Microbiological and Chemical testing laboratory with over 25 years of experience in the Regulatory Testing, Seafood, Shellfish, Meat and Poultry, Production Plant, Environmental, Health Care, Pharmaceutical and Nutritional market place.
    - U.S. Food and Drug Administration registered laboratory.
    - Previously Recognized by USDA, FSIS Listeria and Salmonella Program (#0093).
    - Previously Recognized by USDA, FSIS Chemistry Program (#1282).
    - Centers for Disease Control (CDC) Contract Laboratory, Vessel Sanitation Program.
    - U.S. Food and Drug Administration accepted laboratory for import testing.
    - U.S. Customs accepted laboratory.
    - U.S. Dept. Of the Interior, Nat'l. Parks Contract Laboratory, Everglades, Flamingo and
    - Biscayne National Park Systems.

[redacted]

Kappa Laboratories, Inc.

2577 NW 74 Avenue
Miami, Florida 33122

Mt. Sinai Medical Center
Biomedical Research Building
4300 Alton Road
Miami Beach, Florida 33140

- ENVIRONMENTAL TESTING
  - Florida Department of Health Accredited Laboratory – ISO 17025
    State Laboratory ID: E86515
    State Laboratory ID: E86942
  - Kappa Laboratories is an accredited full-service Microbiological and Chemical testing laboratory with over 25 years of experience in the Environmental, Cosmetic, Health Care, Pharmaceutical and Nutritional areas.
    - Contract Laboratory to the Center for Disease Control Vessel Sanitation Program
    - Contract Laboratory to the National Parks System, South Florida
    - Contract Laboratory for U.S. Coast Guard Alaska
    - U.S. Food and Drug Administration Accepted Laboratory for import testing
    - U.S. Customs Accepted Laboratory

[redacted]

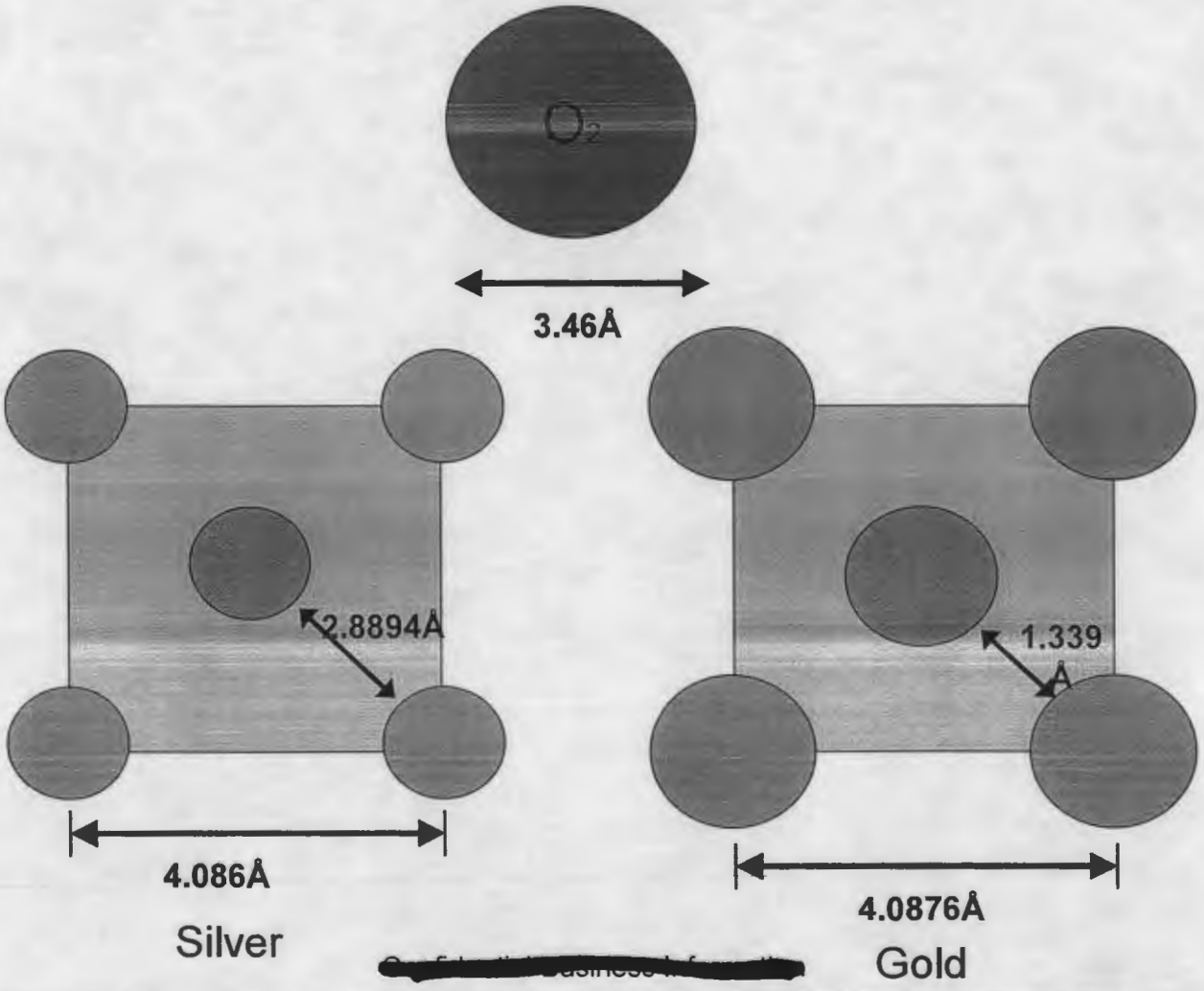
Silver Is Unique Metal In Its Behavior With Oxygen

- Molecular oxygen is absorbed onto the surface of silver as atomic oxygen
- Because atomic oxygen fits into the octahedral holes of silver, oxygen accumulates within the bulk of silver
- This stored oxygen significantly contributes to the catalytic oxidative power of silver
- When pure silver is melted in air, it absorbs about ten times its volume, or 0.3 % of its weight in oxygen
- Industrial application of silver as oxidative catalysis
- Conversion of methanol to formaldehyde, ethylene to ethylene oxide (23,000,000 troy ounces of Ag/year)
- Antiseptic action by atomic oxygen
- Atomic oxygen formed by silver readily oxidizes bacteria or virus, resulting in complete disintegration

20

Molecular Oxygen Interaction

Topology of Silver Versus Gold



Nanosilva™ Antimicrobial
Efficacy Data

- Silver impregnated Nanosilva NSPW-L30SS plastic coupons vs. Control No-Active plastic coupon
- Two (2) to three (3) log E. coli reduction over a 24 to 48 hour period using Nutrient Agar
- Similar results under Non-Nutrient conditions utilizing the same procedures

Nanosilva™ Antimicrobial
Leaching Analysis

- Directly incorporated into plastic matrix
- Non-Leaching from LDPE Polymer as tested
- Resistant to Leaching at pH 2.0 to 10.0
- Resistant to Microbial Leaching – 72 Hrs
- Detection Limit 0.2 PPB by ICP

Nanosilva™ Antimicrobial
Composition and Information on Ingredients

- Chemical Name: Silver-Silica Colloid
- Formula: Ag0.001-0.01µSiO2
- Molecular weight: 60.198 – 61.168
- Components: Silver-Silica (covalently bound), Ethylene Glycol and Water

[redacted]

Nanosilva™ Antimicrobial
Physical Data

- Appearance: Yellow opaque liquid
- Odor: no odor
- Boiling Point: 101 C
- Density: 0.99 To 1.01 mg/ ml
- pH of 1.0% Solution: 2.5 – 3.5
- Volatility: Non-volatile
- Solubility in water: Insoluble in water, dispersed as particles
- Viscosity: 3,800 cps as slurry

[redacted]

Nanosilva™ Antimicrobial Hazards Analysis

- Acute Toxicity: (oral) Acute Oral LD50: 100% survival at 5000 mg/Kg (Mouse)
- Acute Toxicity: (dermal) Acute Dermal LD50: 100% survival at 5,000 mg/Kg (rat)
- Inhalation (acute): LC50 greater than 2.07 mg/L
- Ingestion: Acute Oral LD50 greater than 5,000 mg/Kg (rat)
- Skin: Not a Contact Sensitizer, Buehler Method
- Dermal Irritation: (PDII): 0.3 average score, slightly irritating clear in 72 hrs.
- Eyes (primary irritation): MMTS: 16.7 (Average Irritation Score), Mildly irritating

[redacted]

Nanosilva™ Antimicrobial
Ecological Analysis

- Biodegradation: Degradation will follow the path of elemental compounds.
- Products of Degradation: Non-toxic degradation to basic elemental composition.
- Ecotoxicity: Not needed based on low exposure and use.

[redacted]

Nanosilva™ Antimicrobial
The Promise in Nanotechnovation

- Oxygen free radical formation
- Maximized surface area / reaction
- Product of nanotechnology
- Structural stability
- Low concentration
- Low toxicity
- Non-leaching/non-migrating

Exhibit C.

NanoSilva™ Antimicrobials, Presentation to the EPA Office of Pesticide Programs Nanotechnology Committee, *List of Attendees* (Nov. 8, 2007).

Nano Silva Mtg

308-6341	Marshall/Swindell	EPA/AD/RM 33	Swindell.Marshall@EPA.gov
242-7777	Mark Gustin	Amco Plastics	mgustin@amco.ws
231 308-8169	Betty Shackleford	EPA/AD	Shackleford.Betty@epa.gov
3-305-6450	Tim Dole	EPA/HED	Dole.Timothy@EPA.GOV
3-305-7606	Matthew Crowley	EPA/HED	crowley.matthew@epa.gov
3-305-9096	Tamue Gibson	EPA/FB	gibson.tamue@epa.gov
3-308-0175	Debbie Smegal	EPA/HED	Smegal.Deborah@epa.gov
398-8177	Ben Chambliss	EPA/AD	Chambliss.Ben@epa.gov
3-308-9445	A Najm Shamim	EPA/AD	Shamim.Najm@epa.gov
3-305-7565	Jenny Tao	EPA/AD	Tao.Jenny@epa.gov
3-305-6475	Nathaniel Martin	EPA/FEAD	Martin.nathaniel@epa.gov
605 0525	Lance Cornell	EPA/SRRD	cornell.lance@epa.gov
3080460	Angela Gonzales	EPA/BPPD	gonzales.angela@epa.gov
3088163	Jack Housenger	EPA/HED	housenger.jack@epa.gov
3-305-1049	Bill Jordan	OPP	jordan.william@epa.gov
3-308-8087	Dennis Edwards	OPP/AD	edwards.dennis@epa.gov
308-8362	Demson Fuller	OPP/AD	Fuller.demson@epa.gov
31-293 1602	Gary Metzger	Amco/Nanosilva	gmetzger@amco.ws
40-338-2219	Mary K. Bruch	Consultant Nanosilva	marybruch@magagyis.net
5-549-0199	DR. Peter J. Kaweck	Kappa Laboratories	PJK@KAPPALABS1.com
2-615-4906	Wayne J. Krause	Nanosilva	WKrause@clairson.com
3-[redacted]-4378	Charles Franklin	[redacted] Akin Gump LLP	cfranklin@akingump.com

Exhibit D.

EPA, Office of Pesticide Programs, Notice of Pesticide Registration, AgION® Silver Antimicrobial Type AD Pesticide Label (Aug. 23, 2007).

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Antimicrobials Division (7510C)
1200 Pennsylvania Avenue NW
Washington, D.C. 20460

NOTICE OF PESTICIDE:
X Registration
___Reregistration

(under FIFRA, as amended)

EPA Reg. Number:	Date of Issuance:
72854-1	AUG 23 2007

Term of Issuance: Unconditional

Name of Pesticide Product: AgION Silver Antimicrobial Type AD

Name and Address of Registrant (include ZIP Code):

AgION Technologies Inc.
60 Audubon Road
Wakefield, MA 01880

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Registration Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act.

Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This product is conditionally registered in accordance with FIFRA Section 3(c)(7)(A) provided that you:

1. Submit and/or cite all the data required for registration of your product under FIFRA Section 3(c)(5) when the Agency requires all registrants of similar products to submit such data; and submit required by registration review under FIFRA Section 3(g).

2. Make the following labeling changes:

   a. Revise the EPA Registration Number to read, EPA Reg. No. 72854-1.

   b. Place the appropriate EPA Establishment Number on the product labeling.

Signature of Approving Official:

Marshall Swindell, Product Manager, Team 33
Regulatory Management Branch I, Antimicrobials Division

Date: AUG 23 2007

EPA Form 8570-6

Page 2
EPA Reg. No. 72854-1

3. Submit three copies of the final printed label prior to releasing this product for sale.

The Confidential Statement of Formula dated July 26th, 2007, is acceptable.

Submit a one-year long Storage Stability and Corrosion Characteristics study to the Agency for review.

A stamped copy of the label is enclosed for your records.

If these conditions are not complied with, the registration will be subject to cancellation in accordance with FIFRA sec.6(e). Your release for shipment of the product constitutes acceptance of these conditions.

Sincerely,

Marshall Swindell
Product Manager 33
Regulatory Management Branch I
Antimicrobial Division(7510PC)

3 7 6

AgION® Silver Antimicrobial Type AD

A preservative and bacteriostatic agent for use in the manufacture of polymer, plastic and latex products. For commercial and industrial use only.

Active Ingredient:	
Silver	22.00 %
Other Ingredients	78.00 %
Total	100.00 %

KEEP OUT OF REACH OF CHILDREN

DANGER

SEE INSERT LABEL FOR PRECAUTIONARY STATEMENTS

Manufactured for:
AgION Technologies, Inc.
60 Audubon Road
Wakefield, MA 01880

EPA Registration No. 72854-Y
EPA Establishment No. 72854-MA-001

ACCEPTED
with COMMENTS
In EPA Letter Dated:

AUG 2 3 2007

Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.

72854-1

Net Wt. XXXX

Lot No. XXXXXXXXX

4 7 6

Directions for Use

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

AgION® Silver Antimicrobial Type AD **is an antimicrobial additive to be used by compounding into many polymeric materials. It is designed to be incorporated during the manufacturing process to impart antimicrobial activity to the manufactured products.** ***AgION® Silver Antimicrobial Type AD*** **suppresses the growth of algae, mold, mildew, fungi and bacteria which cause unpleasant odors, discoloration, staining, deterioration or corrosion only. No finished product incorporating** ***AgION® Silver Antimicrobial Type AD*** **may make any public health claims relating to antimicrobial activity without first obtaining an EPA registration or FDA clearance for the finished product which permits such claims, and without a tolerance or exemption from the requirement of a tolerance. When incorporated into treated articles, this product does not protect users of any such treated article or others against food borne or disease causing bacteria, viruses, germs or other disease causing organisms.**

ACCEPTED
with COMMENTS
In EPA Letter Dated:

AUG 23 2007

Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.

72854-I

Types of Finished Products

Plastics - including films, sheets, slabs, and molded plastic parts

The additive may be incorporated into the finished product at up to 5.0% by weight or at least 0.1% for bulk plastics. Contact AgION Technologies, Inc. to determine the appropriate amount of AgION® Silver Antimicrobial Type AD for individual finished products.

Non-food contact uses only:

Medical Devices, Equipment and Supplies

Fibers

The additive may be incorporated into the finished product at up to 5.0% by weight or at least 0.1% for fibers. Contact AgION Technologies, Inc. to determine the appropriate amount of AgION® Silver Antimicrobial Type AD for individual finished products.

Non-food contact uses only:

Medical Devices, Equipment and Supplies

Coatings, Films and Laminates

The additive may be incorporated into the coating, film or laminate applied to the finished product at up to 5.0% by weight, or at least 0.05% for paper or 0.1% for bulk plastics. Contact AgION Technologies, inc. to determine the appropriate amount of AgION® Silver Antimicrobial Type AD for individual finished products. Types of coatings include water-borne, solvent-borne, 100% solids, radiation cure, liquid and powder.

Non-food contact uses only:

Medical Devices, Equipment and Supplies

Adhesives and Sealants

The additive may be incorporated into the finished product at up to 5.0% by weight, or at least 0.05% for paper or 0.1% for bulk plastics. Contact AgION Technologies, Inc. to determine the appropriate amount of AgION® Silver Antimicrobial Type AD for individual finished products.

Non-food contact uses only:

Medical Devices, Equipment and Supplies

Miscellaneous Applications

The additive may be incorporated into the finished product at up to 5.0% by weight or at least 0.1%. Contact AgION Technologies, Inc. to determine the appropriate amount of AgION® Silver Antimicrobial Type AD for individual finished products.

Non-food contact uses only:

Indoor Paints and Coatings

ACCEPTED
with COMMENTS
In EPA Letter Dated:

AUG 23 2007

Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.
72854-1

876

PRECAUTIONARY STATEMENTS

Hazards to Humans: Harmful if inhaled or absorbed through skin. Causes moderate eye irritation. Avoid breathing dust. Avoid contact with skin, eyes or clothing. Wear goggles or face shield and rubber gloves when handling the dry powder. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash clothing before reuse.

FIRST AID	
If on skin or clothing	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15 – 20 minutes. • Call a poison control center or doctor for treatment advice.
If in eyes	• Hold eye open and rinse slowly and gently with water for 15 – 20 minutes. • Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. • Call a poison control center or doctor for treatment advice.
If inhaled	• Move person to fresh air. • If person if not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. • Call a poison control center or doctor for further treatment advice.
If swallowed	• Call poison control center or doctor immediately for treatment advice. • Have person sip a glass of water if able to swallow. • Do not induce vomiting unless told to do so by the poison control center or doctor. • Do not give anything by mouth to an unconscious person.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment.	

Storage and Disposal

Do not contaminate water, food or feed by storage and disposal.

Pesticide Storage: Do not store in areas accessible to children. Keep product dry and containers covered during storage; store below 130°F.

Container Disposal: Inner Plastic Bag: Completely empty plastic bag into application equipment. Then dispose of empty bag in a sanitary landfill or by incineration, or, if allowed by State and local authorities, by burning. If burned, stay out of smoke. **Outer Steel Can:** Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by other procedures approved by State and local authorities.

Pesticide Disposal: Wastes from the use of this product may be disposed of on site or at an approved waste disposal facility.

ACCEPTED
with COMMENTS
In EPA Letter Dated:

AUG 2 3 2007

Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No. 72854-7

Exhibit E.

Agion®, Technology - "How it Works" (2006), http://www.agion-tech.com/Technology.aspx?id=156 (last visited Nov. 28, 2007).

EPA asked if the nanosilver is still there as nano as the life cycle proceeds. Is the color change indicative of change in the nano structure? Also questioned was the molecular weight of the polymer, as well as how much silver is in a coupon compared to what theoretically should be there. Coupons used in the study were all produced from the same batch of polymer.

Wayne discussed label claims with Marshall Swindell and reiterated that he intends to revise the use patterns and claims in the labeling and forward next week for review and comments. Marshall said he will work with Nanosilva on the label.

Additionally, it was discussed that Nanosilva would prepare Waiver Requests for additional Tier 1 Toxicology testing and submit with application for registration of Nanosilva as a new chemical under fee category A420.

Jack Hausinger stated that because this is nanotechnology, the review may take longer as they do not want to make a mistake. Don Sauey responded by describing all the time and studies followed with meetings with EPA to facilitate efforts of EPA to determine their nanotechnology policy while Nanosilva did not submit their application. He stressed that they have done all that EPA asked and the he believes the data supports claims of no leaching of silver from polymers treated with Nanosilva product. He declared Nanosilva's intention to submit their application as a new chemical for treated articles as soon as possible.

EPA Meeting with Nanosilva®
July 7, 2009
EPA Building
One Potomac Yard
Arlington, VA

Attendees for Nanosilva

Don Sauey
Wayne Krause - Nanosilva
Greg Jones
Lee Miller
Dr Seong-Geun Oh - Dean Chemical Engineering, Hanyang Univ. – Seoul, Korea
Dr. Peter Kmieck – Kappa Laboratories, Miami, FL
Mary K Bruch - Micro Reg Consultant

Primary purpose for the meeting was to discuss results of the Leachability Study completed at the request of the OPP Committee and Waiver Requests to be made by Nanosilva for additional Tier 1 Toxicology testing based on the favorable results of the study.

After introductions, Wayne Krause summarized previous (the fifth) meeting between EPA and Nanosilva (March 31, 2008). This meeting was predicated on the EPA decision that nano materials and products would require a new chemical application. Discussions about proposed leachability study were had and guidance was given by EPA members present in the meeting. The chemistry and rationale for the proposed application had been reviewed at the March Meeting. At the conclusion of the meeting in March 2008, Betty Shackelford stated that EPA would consider waivers for the additional sub-chronic and chronic toxicology testing required for a new chemical application provided Nanosilva could show that silver did not leach from treated materials and would limit use of technology to exclude food contact and textile as a minimum. Wayne reiterated the toxicology and chemical testing had already been completed and provided for review previous to the March 31, 2008 meeting.

Following the summary, Wayne introduced Dr. Oh as the inventor of Nanosilva's product and marketer in Korea and other countries. Dr. Oh presented a comprehensive review of the history of silver use. He emphasized the chemistry of the silver/silica particle in the Nanosilva product. He also stressed the strong oxidation in the silver molecule with atomic oxygen. There was discussion of particle size of the product. TEM pictures showed a particle size of 14nm – size range of nano-particles was 3-70 nm. When applied in textiles, it appears that the color varies with the size of the particles (EPA chemist observed). Dr. Oh also listed the many applications in many products marketed in Korea, India and Japan. Dr. Oh emphasized again that the antimicrobial action of the Nanosilva particle in materials is from oxidation reactions and not the same as silver-ion exchange activity in which the silver ions must be released from a surface to be effective.

Dr. Peter Kmieck presented an extensive study based on an FDA protocol to evaluate leaching from LLDPE coupons with Nanosilva incorporated as a final product. He described the analytical procedures used for the 5g coupons exposed to varying solvents, abrasion and temperatures. One EPA chemist asked what the silver mass content in a 5g coupon would be. Several difficulties in measurement were described, e.g., alcohol at high temperatures and background readings from test materials. The limit of detection was 2ppb. A questioner from